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North Central Forest Experiment Station

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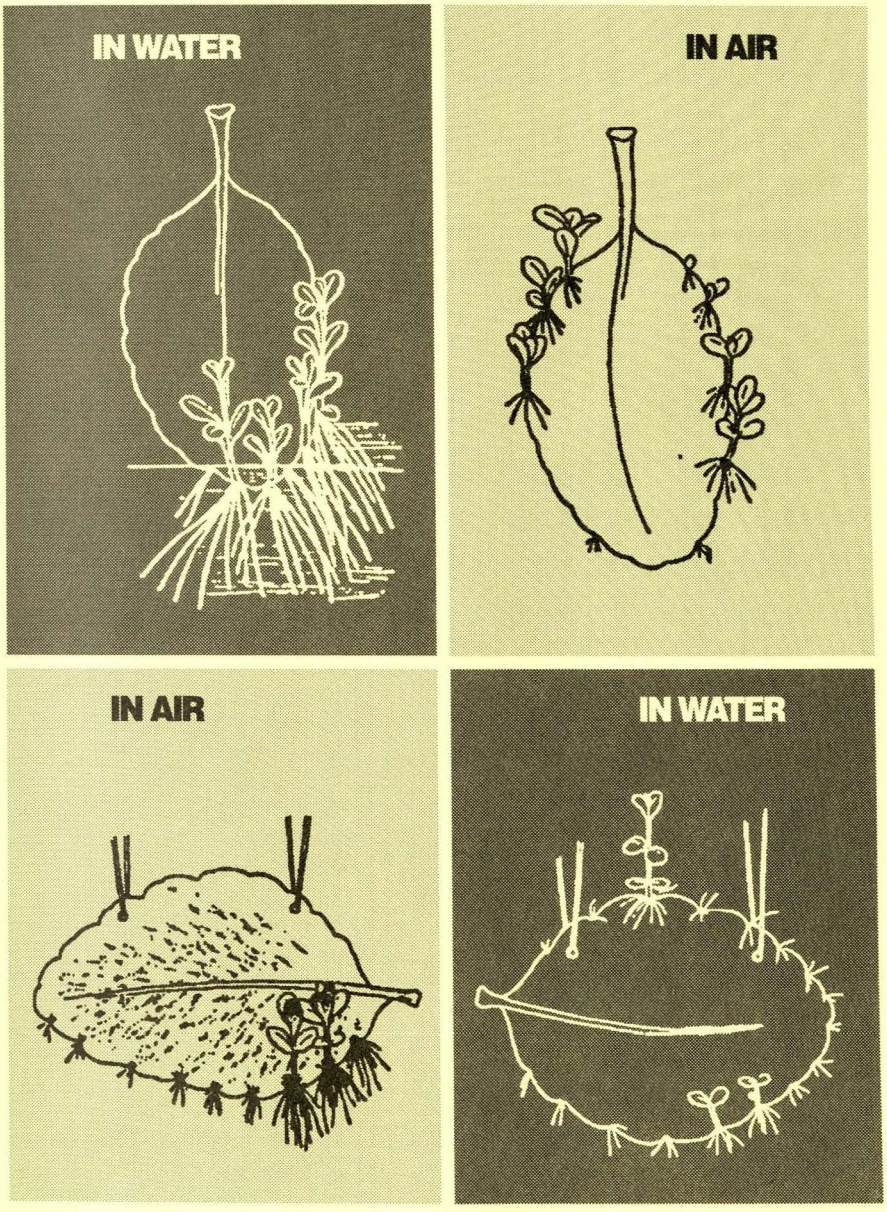
Abstracts of Presentations at the First International Symposium on the Biology of Adventitious Root Formation



COVER GRAPHICS: Upper figure: Adventitious roots and shoots regenerate in different places when a *Bryophyullum* leaf is suspended entirely in air (right leaf), compared to dipping the apex into water.

Lower figure: Adventitious roots and shoots regenerate only in the notches of the lower side of a *Bryophyllum* leaf suspended in air (left leaf), and a red pigment develops only in such leaves. This "directed influence of gravity" does not exist when a leaf is wholly suspended in water. [Illustrations from Jacques Loeb. 1924. *Regeneration from a Physico-Chemical Viewpoint*, New York, NY: McGraw-Hill Book Co.: 21 and 27.]

North Central Forest Experiment Station
Forest Service—U.S. Department of Agriculture
1992 Folwell Avenue
St. Paul, Minnesota 55108
1993
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PREFACE

The Symposium provides the first international forum for the discussion of past research progress and future research directions in botany, quantitative and molecular genetics, biochemistry, biophysics and plant physiology concerning *the biology* of adventitious root formation. Historically, interests in the biology of adventitious root formation have ranged from academic studies within plant morphogenesis and ecology to empirical trials to improve cloning of special genotypes for agronomy, forestry, and horticulture research and practice. Thus, the Symposium serves all such special interests in one way or another.

The Symposium offers participants invited lectures (19 speakers with 14 coauthors from eight countries), contributed posters (about 75 with about 170 authors from 26 countries), and formal discussions by symposium participants—and scientific socials, luncheons, a dinner and a post-conference tour. The Program was designed to facilitate the free and open exchange of scientific and technical information among the world's most competent and interested researchers of what can be generalized as the biology of "adventitious rooting."

The invited lectures of the Symposium will be published in final form in *The Biology of Adventitious Root Formation*, T.D. Davis and B.E. Haissig (eds.), Plenum Press, New York. Members of the Symposium Organizing Committee also served on the Editorial Advisory Board for the book. Each symposium participant will automatically receive a copy of the book; for additional copies write to Plenum Publishing Corporation, 233 Spring Street, New York, NY 10013-1578.

The members of the Executive Committee and of the Organizing Committee have enjoyed developing the Symposium because it will foster research on the internationally important subject of adventitious root formation. Thus, both committees thank our host and our financial sponsors for their willing and generous support of this scientific endeavor. The committees also thank all those individuals who, in the background and without due recompense, made the Symposium possible through their diligent efforts.

Bruce E. Haissig
Tim D. Davis
March 1993

Abstracts of Platform Presentations and Posters

The First International Symposium On The Biology Of Adventitious Root Formation

Dallas, Texas April 18-22, 1993

Edited by Bruce E. Haissig and Tim D. Davis

Host

Texas A&M University Research and Extension Center

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PROGRAM

The First International Symposium On The Biology Of Adventitious Root Formation

Sunday, April 18

2:00-6:00 pm • Registration at the Hotel

6:00-7:30 pm • Welcoming Reception at the Hotel

Monday, April 19

8:00 am • Registration Reopens

Symposium Opening

9:00 am • Opening remarks by Bruce E. Haissig, Chairman, Executive Committee

9:15 am • Welcome by James A. Reinert, Resident Director of Research, Texas A&M University, Dallas, USA

9:20 am • Welcome by R. Daniel Lineberger, Head, Department of Horticultural Science, Texas A&M University, College Station, USA

9:30 am • Introduction to the Symposium by Tim D. Davis, Chairman, Organizing Committee

9:40 am • Keynote Address by Charles E. Hess, University of California at Davis, USA, "The Art and Science of Adventitious Root Formation"

10:30 am • Break

Special Lecture

11:00 am • "Origin, Biology and Diversity of Shoot-Borne and Adventitious Roots" by Peter W. Barlow, University of Bristol, UK

11:45 am • Lunch

Session Number One

The Commercial Importance of Adventitious Rooting

Moderated by Dale E. Kester, University of California at Davis, USA

1:00 pm • "Agronomy" by John L. Kovar, Louisiana State University, Baton Rouge LA, USA

1:30 pm • "Forestry" by Gary Ritchie, Weyerhaeuser, Centralia WA, USA

2:00 pm • "Horticulture" by Fred T. Davies, Jr, Texas A&M University, College Station, USA

Session Number Two

Infrastructure for Research

Moderated by Narendra Sankhla, J.N. Vyas University, Jodhpur, India

2:30 pm • "Genomic Manipulation of Plant Materials for Adventitious Rooting Research" by Don E. Riemenschneider, US Forest Service, Rhinelander WI, USA

3:15 pm • Break

3:45 pm • "Model Systems for Studying Adventitious Rooting" by Stephen G. Ernst, University of Nebraska, Lincoln, USA

4:30 pm • Panel Discussion on "The Infrastructure for Adventitious Rooting Research" moderated by Johannes van Staden, University of Natal, South Africa

5:00 pm • Adjourn

7:30-9:00 pm • **First Poster Session** with all authors present

Tuesday, April 20

Session Number Three

Setting the Stage for Rooting

Moderated by Wesley P. Hackett, University of Minnesota, St. Paul, USA

8:30 am • "Novel Experimental Systems for Determining Cellular Competence and Determination" by Debra Mohnen, University of Georgia, Athens, USA

9:15 am • "Differential Competence of Histologically Similar Cell Types in the Juvenile and Mature Phases of *Hedera helix*" by John R. Murray, University of Minnesota, St. Paul, USA

10:00 am • Break

10:30 am • "Biochemical and Molecular Markers for Cellular Competence" by Paul Hand, Horticulture Research International, Littlehampton, UK

11:15 am • "Manipulating Rooting Potential" by Brian H. Howard, Horticulture Research International, East Malling, UK

12:00 • Lunch

Session Number Four

Induction of Rooting

Moderated by Arie Altman, Hebrew University, Rehovot, Israel

1:15 pm • "Role of Plant Growth Regulators and the Environment" by A. Skytt Andersen, Royal Veterinary and Agricultural University, Copenhagen, Denmark

2:00 pm • "Auxin and Adventitious Root Initiation" by David Blakesley, University of Bath, Bath, UK

2:45 pm • "The *ERabp* Gene Family: Structural and Physiological Analyses" by Klaus Palme, Max Planck Institute, Cologne, Germany

3:30 pm • **Second Poster Session** with all authors present

4:30 pm • Panel Discussion on "The Applications of New Biotechnologies to Rooting Research" moderated by Steve Chandler, Calgene Pacific Pty Ltd, Collingwood, Australia

5:00 pm • Adjourn

6:00-9:00 pm • **Social and banquet at the hotel with speaker Jerry M. Parsons**, Texas Agricultural Extension Service-San Antonio, who will discuss the need for rooting research from a horticultural industry perspective.

Wednesday, April 21

Session Number Five

Root System Development and Plant Growth

Moderated by Judson G. Isebrands, US Forest Service, Rhinelander WI, USA

8:30 am • "Use of Root Culture for Gene Expression and Root Development Studies" by John D. Hamill, Monash University, Melbourne, Australia

9:15 am • "Biochemical and Molecular Genetic Control of Root System Architecture" by David Tepfer, INRA, Versailles, France

10:00 am • Break

10:30 am • "Root System Development in Rice" by Shigenori Morita, University of Tokyo, Japan

11:15 am • "Modelling Root System Development: Clonal Poplars" by Donald I. Dickmann, Michigan State University, East Lansing, USA

12:00 • Lunch

1:15 pm • "The Role of Expert and Hypertext Systems in Modelling Root-Shoot Interactions and Carbon Allocation" by H. Michael Rauscher, US Forest Service, Grand Rapids, MN, USA

2:00 pm • "Carbon Allocation to Root and Shoot Systems of Woody Plants" by Alex Friend, Mississippi State University, Mississippi State, USA

2:45 pm • Panel Discussions

"Coupling Molecular Level Studies to Biochemistry and Physiology" moderated by Thomas Gaspar, Universitéde Liège, Belgium.

"Specific Root-Inducing Substances" moderated by Ernesto Vieitez, Catedrático de Universidad, Santiago de Compostela, Spain

"Rooting Terminology" moderated by Shigenori Morita, University of Tokyo, Japan

Thursday, April 22

Post-Conference Tour

8:30 am - 3:00 pm • Visits to the Texas A&M Research and Extension Center at Dallas, Dallas Arboretum and Botanical Gardens, and City of Dallas overview—organized and led by Tim D. Davis, leaving from and returning to the hotel.

INVITED LECTURES

SPECIAL LECTURE

ORIGIN, BIOLOGY, AND DIVERSITY OF SHOOT-BORNE AND ADVENTITIOUS ROOTS

Peter W. Barlow, Department of Agricultural Sciences, University of Bristol, Long Ashton Research Station, Bristol, BS18 9AF, United Kingdom

Adventitious roots, or more precisely 'shoot-borne' roots, are, in evoluntionary terms, an ancient class of organ, preceding the evolution of the embryonic, or 'pole-borne,' class of roots. Adventitious roots form part of a fundamental iterating developmental unit, the metamer, which consists of leaf, internode, and root. This can be seen even in fossil plants. The suggestion is made that the root originated as a result of the increasing photosynthetic efficiency of the metamer and is evidence of the need of the plant to rid itself of excess carbon. To the advantage of the plant, this excess carbon was fashioned into a cellularised organ that currently often functions in solute capture and in mechanical support for the plant. The diverse forms of adventitious roots in extant plants are related to modifications of their solute capture and support functions by an organ which otherwise might have been simply an elaborate excretory product, as indeed it still is in the case of plants that shed their roots. Examples will be presented of how the various forms of adventitious roots perform these functions and how their position on the plant confirms their presumed evoluntionary origin. Also described will be the place of shoot-borne roots in the ontogeny of the whole plant and how this relates to the concepts of allorhizy and homorhizy as proposed by Goebel and modified by Groff and Kaplan (*Bot. Rev.* 54, 387, 1988).

COMMERCIAL IMPORTANCE OF ADVENTITIOUS ROOTING: AGRONOMY

John L. Kovar, Department of Agronomy, Louisiana State University, Baton Rouge, LA 70893 U.S.A. and *Rolf O. Kuchenbuch*, Institute of Plant Nutrition, Technical University, München, Freising-Weihenstephan, Germany

Agronomic crops in which adventitious root formation is important can be divided into two broad categories—those that are vegetatively propagated and those for which final yield, whether fruit or dry matter, is influenced by the presence of adventitious roots. The number of agronomic species in the first category is small, with sugarcane (*Saccharum* spp.) being the most significant. The second category contains species that play an important role, both directly and indirectly, in world food supply, including rice (*Oryza sativa* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and other Gramineae. Current research on adventitious root formation and function in agronomic species is quite broad, ranging from the cellular level to field level. Major areas of study include the efficiency of water and ion absorption and transport, effects of environmental conditions on optimal growth and development, and responses to environmental stress. Additional research is needed on the mechanisms that control partitioning of nutrients and assimilates during the plant life cycle, as well as the influence of environmental conditions on these control mechanisms. A better understanding of the effectiveness of adventitious roots in absorption of water and nutrients is necessary before genetic engineering can be used to "design" plants with efficient root systems.

COMMERCIAL IMPORTANCE OF ADVENTITIOUS ROOTING: FORESTRY

Gary A. Ritchie, Weyerhaeuser Company, G.R. Staebler Forestry Research Center, Centralia, WA 98531 U.S.A.

Forestry is, and always has been, a seedling-based enterprise. Of the many hundreds of millions of young forest trees planted annually worldwide relatively few are derived from propagation systems dependent on adventitious rooting. However, this is rapidly changing. For various reasons, adventitious rooting is playing an increasingly important role in forest planting stock production.

Use of rooted cuttings in forestry is primarily aimed at capturing two major opportunities. The first is bulking up valuable seed supplies from: (a) scarce provenances, (b) valuable species which are difficult to propagate by seed, and (c) seed produced in genetic improvement programs. The second, more powerful, application is cloning unique and highly valuable genotypes.

Production technology is generally designed to arrest maturation of donor plants and may include hedging, serial propagation, or juvenile stock plant systems. Rooting technology has not advanced appreciably in the recent past and often employs indoor systems with mist or fog propagation and bottom heat. Plants are delivered to the field either as bareroot or container stock depending on the systems in use for seedling production. These technologies have been applied to broad-leaved trees such as cottonwood, willow, and eucalyptus for many decades and to the conifer *Cryptomeria japonica* for many centuries. Within the past 15 years techniques have been developed for other commercial conifers as well, primarily spruces, pines, and Douglas-fir.

The major research challenges involve understanding and manipulating donor plant maturation state, and developing system-wide efficiencies to bring production costs in line with those associated with seedling production.

COMMERCIAL IMPORTANCE OF ADVENTITIOUS ROOTING: HORTICULTURE

Fred T. Davies, Jr., Department of Horticultural Science, Texas A&M University, College Station, TX 77843-2133 U.S.A. and *Tim D. Davis*, Texas A&M Research & Extension Center, Dallas, TX 75252-6599 U.S.A.

Clonal propagation is commonly used with the commercial production of many horticultural crops (ornamentals, fruit crops, and vegetables). Over 75% of the propagation systems used in the ornamental horticulture industries (nursery crops, greenhouse, floriculture, foliage plants) depend on successful rooting of cuttings. In the USA alone, grower cash receipts and consumer expenditures for ornamental crops were $8.9 billion and $40 billion, respectively, for 1991. The USA export value of horticulture crops exceeds all other agriculture commodities, including forestry and agronomic crops.

Successful rooting continues to be a serious limitation to the asexual propagation of many woody horticultural crops. Many costly and labor-intensive systems are employed to improve rooting success such as mounding/stooling, layering, division, separation, graftage, budding, tissue culture, manipulation of stock plants through etiolation and banding, etc. Labor costs contribute to 30-65% of nursery crop production costs, and more than 80% of propagation expenditures. Hence, there is considerable financial incentive to streamline propagation techniques and improve rooting success. Successful rooting of recalcitrant species will lead to the development of new plant products and markets. The difficulty in rooting recalcitrant species and in developing unique clones opens up new opportunities for utilizing biotechnology, such as gene manipulation, to increase tissue sensitivity to auxin and other limiting rooting factors, as well as improving tissue culture systems. Successful rooting and acclimation of tissue culture produced plants will need to be improved if biotechnology is to be commercially incorporated into the propagation and production of horticultural corps.

GENOMIC MANIPULATION OF PLANT MATERIALS FOR ADVENTITIOUS ROOTING RESEARCH

Don E. Riemenschneider, U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station, Rhinelander, WI 54501 U.S.A.

Assessment of plant materials, from a genetics viewpoint, is important to the extent that genes affecting a trait of interest can be identified and targeted for molecular manipulation or subjected to artificial selection. Manipulation or selection of genes for adventitious rooting ability is complex because evidence suggests that rooting is a quantitative trait. This paper explores the implications of a quantitative genetic model to provide guidelines for the improvement of rooting using genetic engineering or quantitative genetic technologies. The *de facto* goal of genetic engineering for rooting is to increase the activity of one or a few enzymes belonging to a rooting-related biochemical pathway. Hopefully, increased enzyme activity will result in a total pathway flux that exceeds some threshold value beyond which a rooting phenotype is expressed. Application of metabolic control theory suggests that engineering approaches will be successful when the product (enzyme) of a modified gene has a very large control coefficient or has a regulatory effect on a large number of rooting-related genes. Thus, molecular studies should target genes whose products possess the aforementioned properties. An alternative to genetic engineering is the application of artificial selection within a breeding population. Standard biometrical plant breeding tools are readily applied to the improvement of rooting when multiple observations can be made on each genotype in the breeding population. In such cases the selection criterion, "percent rooted cuttings," becomes quasi-normal. But, multiple observations are not always possible. Then, individuals are often classed only as "rooting" or "non-rooting" and the selection criteria is a threshold trait. The theory of breeding for threshold traits suggests that selection is maximally efficient only when the proportion of individuals in the rooting class equals the selection intensity. Thus, adjustment of experimental conditions to alter rooting percentage or the use of indirect selection criteria may be needed.

MODEL SYSTEMS FOR STUDYING ADVENTITIOUS ROOTING

Stephen G. Ernst, University of Nebraska, Lincoln, NE 68583-0814 U.S.A.

Model systems have been a driving force in biological research for decades. From *E. coli* and associated phages to *Neurospora*, yeast, *Drosophila*, *C. elegans*, mouse, etc. Relatively recently *Arabidopsis* has become the dominant model system in plant biology. However, several other model systems exist or are being developed to better study a given physiological or developmental pathway or process as specified by the user. In addition to biological considerations, the ability to get funding with a particular model system also drives this process. The purpose of this manuscript is to promote discussion regarding the selection of model systems for plant biology research. In particular, the discussion will focus on: (1) the rationale that results in certain model systems becoming prominent in plant biology research today, (2) a brief assessment regarding how well knowledge and technology is transferred to applied species or "higher order" models, (3) the need for manipulable homogeneous plant cell lines for the next order of magnitude in plant biology research at the molecular and biochemical level with application in signal transduction and developmental biology, and (4) current and future model systems for studying adventitious rooting.

NOVEL EXPERIMENTAL SYSTEMS FOR DETERMINING CELLULAR COMPETENCE AND DETERMINATION

Debra Mohnen, Complex Carbohydrate Research Center and Department of Biochemistry, University of Georgia, Athens, GA 30605-2612 U.S.A.

Adventitious roots form at locations in the plant at which roots are not normally found, i.e., locations other than the embryo or the primary root. The formation of roots within foreign tissues requires that a cell or group of cells within the tissue embark upon a new developmental program. Studies with *in vitro* cultured tissue explants have identified two operationally defined early stages of adventitious root organogenesis: competence and determination (1). Tissue explants have been particularly useful for studying the developmental stages of root organogenesis because their physical separation from the rest of the plant allows transfer of the tissues to various media. Competence of tissue explants for root formation refers to the ability of cells within the tissues to respond to specific root-inducing stimuli (e.g., auxin and cytokinin) by the formation of roots. Once competent tissues have been exposed to an inducer the tissues may become determined for root formation, i.e., the tissues continue root organogenesis in the absence of the inducer. The goal of this paper is to summarize the current state of knowledge regarding competence and determination of tissue explants for root organogenesis. Emphasis will be placed on systems using *in vitro* cultured explants including leaf explants (1,2) and thin cell-layer explants from flower branches (3). The merits and limitations of these systems for understanding root organogenesis will be discussed.

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2. Attfield, E.M.; Evans, P.K. 1991. *J. Exp. Bot.* 42: 59-63.
3. Mohnen, D.; Eberhard, S.; Marfa, V.; Doubrava., N.; Toubart, P.; Gollin, D.; Gruber, T.; Nuri, W.; Albersheim, P.; Darvill, A. 1990. *Development* 108: 191-201.

DIFFERENTIAL COMPETENCE OF HISTOLOGICALLY SIMILAR CELL TYPES IN THE JUVENILE AND MATURE PHASES OF *HEDERA HELIX*

John R. Murray, Conchi Sanchez, Alan G. Smith, and *Wesley P. Hackett,* Department of Horticultural Science, University of Minnesota, St. Paul, MN 55108 U.S.A.

The differentiation and histology of cell types of shoot tissue formed during the juvenile phase of *Hedera helix* are surprisingly similar to cell types of tissue subsequently formed during the mature phase, considering the marked difference in the phenotypes of juvenile- and mature-phase tissue. However, these similar cell types of each phase of maturation can have differential competence to express developmental processes. Phloem and inner cortical parenchyma, and duct epithelial cells of juvenile-phase petioles cultured *in vitro* undergo divisions and the formation of adventitious roots in response to auxin. With similar treatment, these cell types of mature-phase petioles produce only callus and do not initiate roots. This difference in competence to form root meristems in juvenile- and mature-phase petioles is inversely related to the localized expression of a proline rich glycoprotein (PRP) gene. Chronologically young mature phase petioles show low levels of expression of the PRP gene in response to auxin treatment and do form adventitious roots. In contrast to this inverse relationship between expression of the PRP gene and competence, the lack of competence to accumulate anthocyanin pigment in a subepidermal cell type of stems and petioles, and mesophyll cells of leaf laminae of mature phase tissue is due to the lack of expression of the dihydroflavonol reductase (DFR) gene, which encodes an anthocyanin biosynthetic enzyme. This lack of competence to express the DFR gene in mature-, but not juvenile-phase tissue, is regulated at the level of transcription. Hypotheses related to the basis of maintenance of differential competencies to express developmental processes during the protracted phases of maturation will be discussed.

BIOCHEMICAL AND MOLECULAR MARKERS FOR CELLULAR COMPETENCE

Paul Hand, Department of Molecular Biology, Horticulture Research International, Littlehampton, West Sussex, BN17 6LP, United Kingdom

Adventitious rooting involves widespread and complex changes in the plant cells affected. Wounding usually precedes root initiation, but rooting does not inevitably follow wounding, especially in mature woody plants. Rooting can be promoted by treatment with auxin and a wide range of other substances.

Our knowledge of the molecular and biochemical factors which underlie and regulate this complex process is extremely limited. Nevertheless there have been some recent exciting advances which suggest that this situation may soon change. These will be summarized and examples given of potential markers of competence which have been identified. Finally, the use of the tools of molecular biology to answer fundamental questions in this area will be discussed.

MANIPULATING ROOTING POTENTIAL

Brian H. Howard, Propagation Science Section, Horticulture Research International, East Malling, West Malling, Kent ME19 6BJ, United Kingdom

Results from recent studies of severe pruning (hedging), and the production of stockplants via micropropagation, question conventional wisdom as to why these treatments enhance adventitious rooting in hardwood cuttings. It is not correct to assume that these are 'rejuvenating' processes simply because increased rooting, shoot vigour and associated characteristics reflect the true juvenile condition. Critical analysis, and the removal of environmental bias towards particular cuttings, show that within these preferred cutting sources it is the subordinate thin shoots that have the highest rooting potential, so any benefits of enhanced vigour are, at most, indirect. There is evidence that rooting potential in hardwood cuttings is predetermined by shoot developmental processes during the preceding growing season, which may involve interactions between shoots at different positions within the hedge. Although cutting thickness is often confounded with shoot position, its effect on rooting appears to be secondary, and independent of any interplay between shoots. Improved rooting in softwood cuttings following dark-preconditioning (etiolation) is also mediated via the development of thinner-than-normal stems. Here, at least one factor appears to be a reduction in carbohydrate demand for stem maintenance to the benefit of rooting. Deficiencies in procedures and in the interpretation of past work are discussed, and hypotheses are proposed to explain rooting potential in softwood and hardwood cuttings which account for the need to apply exogenous auxin, but without embracing long-held views on juvenility and rejuvenation.

ROLE OF PLANT GROWTH REGULATORS AND THE ENVIRONMENT

Lise Bertram and *Arne Skytt Andersen*, Royal Veterinary and Agricultural University, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark

A critical review of the recent knowledge which has been accumulated on the subject will be presented. This will include a status assessment of the research on growth regulators. On this subject significant results have also been obtained regarding the endogenous regulators in the conventional groups, as for some exogenous, synthetic regulators. The growth regulator status and the tissue sensitivity towards growth regulators are probably dependent on environmental conditions. It is well known that the environment which surrounds the stock plants, as well as that to which the cuttings is subjected, is important for the events taking place during root initiation. Furthermore, the interactions among all these factors are, to say the least, complex and interesting both from a theoretical and practical point of view.

The concept of growth regulator function has changed dramatically during recent years and this has implications for our understanding of the involvement of growth regulators in the processes of root initiation in cuttings. Still a number of gaps exist in the knowledge especially regarding the transformation of environmental signals via endogenous regulators into stimuli for the initiation of roots.

AUXIN AND ADVENTITIOUS ROOT INITIATION

David Blakesley, School of Biological Sciences, University of Bath, Bath BA2 7AY, United Kingdom

The fact that endogenous auxin is involved in adventitious root initiation is well established. Much of the evidence to support this notion, collected over many years, is circumstantial. However, there have been a number of recent studies which have used unequivocal physico-chemical techniques to critically measure the levels of endogenous indole-3-acetic acid (IAA) in the rooting zone of cuttings. Several workers have analysed IAA and its metabolites during the period of induction of adventitious root primordia, and subsequent early cell divisions and differentiation.

Agrobacterium rhizogenes causes abundant root initiation at the site of inoculation. Ri plasmid T-DNA contains several genes which encode enzymes involved in the biosynthesis and metabolism of IAA. Controlled expression of these genes in the plant genome potentially offer a further insight for developmental plant physiologists into the role of auxin in the process of adventitious root initiation.

An hypothesis concerning the importance of absolute levels of IAA during adventitious root initiation will be presented, based on data collected from studies described above. The direction of future work in this area of adventitious root biology will be discussed.

THE *ERabp* GENE FAMILY: STRUCTURAL AND PHYSIOLOGICAL ANALYSES

Klaus Palme, *Thomas Hesse*, and *Christine Garbers*, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Germany; and *Carl Simmons* and *Dieter Söll*, Department of Molecular Biology and Biochemistry, Yale University, New Haven, CT U.S.A.

Phytohormones play an important role in coordinating plant growth and development. Determining their mechanisms of action will be a major contribution to our understanding of plant biology. Among the major classes of phytohormones (cytokinins, auxins, gibberellins, abscisic acid, ethylene), auxins are arguably the most important because they control more fundamental and diverse aspects of plant physiology and development. Auxins function throughout the plant by coordinating growth, differentiation and tropisms; by promoting rapid elongation growth, cell proliferation, vascular tissue differentiation and root initiation; and by inhibiting abscision, senescence and bud initiation. Here we describe recent efforts to identify cellular targets of auxin action. We summarize molecular and physiological processes in the characterization of ER-located auxin binding proteins encoded by the *ERabp* gene family. The proteins encoded by this gene family are thought to play a role in auxin perception.

USE OF ROOT CULTURE FOR GENE EXPRESSION AND ROOT DEVELOPMENT STUDIES

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Aseptic root culture systems, in particular those transformed by *Agrobacterium rhizogenes*, have become popular in recent years as a tissue culture system to study metabolism and gene expression in roots. In this presentation we consider the question of how similar these cultures are to the roots of intact plants despite the obvious differences in environment and plant physiology. Examples of specific gene expression, secondary metabolism studies and interactions with root pathogens and symbionts, using experimental data and published information are used to consider this question. Data from experiments involving transformation of *Eucalyptus* species with *A. rhizogenes* will also be presented to consider the respective roles of the plant genotype, the genetic constitution of the *A. rhizogenes* strain which is used and the culture conditions in enabling growing root cultures to be established *in vitro*. In addition we will review examples of root specific gene expression in normal and in transgenic plants with the aim of identifying genes of importance in controlling root development.

BIOCHEMICAL AND MOLECULAR GENETIC CONTROL OF ROOT SYSTEM ARCHITECTURE

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Root systems develop according to the needs of the plant and the availability of resources. This adaptive growth is probably due to differences in branching and elongation. For example, similar elongation in all ramifications will produce a shallow, dense root system. If, for a similar branching frequency, elongation is reduced in all ramifications but one, a principal or tap root will be produced. The regulation of branching, elongation, and thickening in root systems is not understood. It is clear, however, that root systems form in response to environmental signals, coming both from the rhizosphere and from the plant. To simplify the study of root system development we used an experimental model comprised of excised tobacco roots in liquid culture. Thus, signals coming from the aerial portions of the plant were eliminated and the environment was a defined chemical medium. In this system the *rol* A gene (ORF 10) from the Ri TL-DNA of *Agrobacterium rhizogenes*, under the control of the 35S CaMV promoter, increased biomass accumulation and converted a highly branched root system into a tap-root system. These effects were mimicked by inhibiting putrescine accumulation using the specific, irreversible inhibitor, ∝-DL-difluoromethylornithine (DFMO). Simultaneous treatment with putrescine and DFMO abolished the effect of DFMO. We conclude that polyamine metabolism is important in regulating root system architecture and suggest that *rol* A controls root development through polyamine metabolism. DFMO and *rol* A have the potential of increasing root growth *in vitro*, and *rol* A might be useful, *in vivo*, in establishing deeper root systems and, thus, accrued drought tolerance.

ROOT SYSTEM DEVELOPMENT IN RICE

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In rice one seminal root comes out first followed by the emergence, elongation and branching of adventitious roots. Root development proceeds to keep pace with the successive leaf unfoldings. Total number and length of adventitious roots increase to reach their maximum values at around the heading stage. A rice root system at the harvest stage has hundreds of adventitious roots with thousands of lateral roots. The adventitious roots display plagiogravitropism and elongate in various directions. Most parts of the root system distribute in the top 30 cm of soil. Rice is characterized having a shallow and compact root system compared with other cereal crops. A root length density model was constructed on the three simple assumptions: (1) adventitious roots elongate uniformly, (2) root branching is constant along any root axis, and (3) rooting zone is hemispheric. Using the model, the simulation was conducted to determine the average length of adventitious roots and branching degree so that the root length densities fit best. The results of the simulation showed that the estimated indices regarding root system dimensions were less in the shaded plot than in the control plot, which suggested the validity of the root length density model including three simple assumptions.

MODELING ROOT SYSTEM DEVELOPMENT: CLONAL POPLARS

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Species and hybrids within the Aigeiros and Tacamahaca sections of the genus *Populus* generally produce abundant adventitious roots on stem cuttings, a major factor in the worldwide use of poplars in clonal plantation forestry. Modeling must account for morphological characteristics of the poplar root system, seasonal dimensional and biomass growth, and mortality. Morphologically, poplar root systems typically have a strongly developed taproot, which derives from the planted cutting, with strongly developed lateral roots growing away from it. Fine roots (≤1 mm in diameter), while most abundant in the upper soil horizons, can also be found quite deep. However, the physical and chemical properties of the soil can substantially modify the structure and distribution roots. Published data on fine root biomass of poplars is very inconsistent, with estimates ranging from >80% to <5% of total root biomass; interclonal variation accounts for some of the inconsistencies. Fine root production peaks early in the growing season, while mortality occurs mostly in late summer and early fall; both are closely linked to shoot phenology and environmental fluctuations. Temporal differences in fine root turnover are probably due to variation in root production rather than root mortality. Growth of coarse (structural) roots also peaks in the early growing season, but mortality is much lower than with fine roots. Current photosynthate flows to roots throughout the season, but increases substantially after buds set on primary shoot axes. High levels of soil fertility and water decrease the allocation of biomass to root systems. Total non-structural carbohydrate (TNC) and nitrogen reserves in roots reach a low early in the growing season, then build to seasonal highs during the late fall. As much as 50% of root biomass may be reserves. Respiration probably consumes I5% to 30% of root TNC. Though incomplete, current knowledge of root system structure and dynamics can be the basis for constructing the below-ground component of process-based growth models for poplars and other tree taxa.

THE ROLE OF EXPERT AND HYPERTEXT SYSTEMS IN MODELING ROOT-SHOOT INTERACTIONS AND CARBON ALLOCATION

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Knowledge management, and modeling theory and technology have recently matured to provide powerful methods for the entire scientific process. This technology is reviewed and its application to research is explained. An arbitrary theory of root-shoot interaction, taken from the literature, is used as an illustrative example. The example theory is described and then rendered into a HyperText format. Expert system rules are extracted from the HyperText version and then formulated so that they can be implemented in an expert system shell. Finally, the expert system is conceptually placed within a whole plant ecophysiological process model of hybrid poplar called ECOPHYS. My presentation ends with a discussion of the positive and negative aspects of using these methods in the scientific research process.

CARBON ALLOCATION TO ROOT AND SHOOT SYSTEMS OF WOODY PLANTS

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Successful establishment and survival of adventitiously rooted woody-plants is dependent, in part, upon carbon allocation and accumulation in response to plant and environmental factors. Plant control over allocation is influenced by the phenological state, including annual growth cycles, and by long-term development, including differences between the seedling or plantlet life form and that of a reproductively mature tree. Allocation is also affected by environmental factors such as temperature, water, nutrients, aeration and air pollutants. Within the ranges set by genetic, phenological and environmental controls, carbon allocation is normally balanced between root and shoot. This balance depends upon resource availability, root and shoot activity, and is thought to be controlled by the ratio of internal carbon and nitrogen, but may also be mediated by vascular transport of plant growth substances. In adventitiously rooted plants, once the root-shoot balance has been equilibrated, there appear to be few differences in carbon allocation patterns that can be distinguished from those of natural seedlings. However, there is less known about the carbon allocation processes involved in the adventitious rooting of cuttings. Current literature and recently developed techniques, which may assist in answering such questions on the role of carbon allocation in adventitious root dynamics, will be discussed.

SETTING THE STAGE FOR ROOTING

POSTER ABSTRACTS

THE EXPRESSION OF *AGROBACTERIUM RHIZOGENES ROL* B GENE IN TOBACCO RHIZOGENIC EXPLANTS

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Rol B has been shown to be the only gene of the T-DNA from *Agrobacterium rhizogenes* capable alone of inducing root differentiation in plants. In order for rol B to direct rhizogenesis, both auxin and a long non-coding region at the 5' of the gene are needed. We introduced rol B and its promoter into tobacco plants. Rol B expression was visualized using gene fusions of the rol B promoter with the GUS gene of *E. coli.* Thin layers of superficial transgenic tissues and of controls were cultured on a rhizogenic medium (Torrigiani *et al.* Physiol. Plant. 77: 294-301, 1989). The number of explants with roots and the mean number of roots per explant was significantly greater for rol B explants as compared to the control. Similarly, the number of root meristemoids observed in transgenic explants was greater than that of the control. GUS staining was observed during the endocytogenic growth phase that preceded root meristemoid organization. Only some of the endocytogenic areas were stained, and within the same area, groups of both stained and unstained cells were present. Only meristemoids and root primordia formed in the areas of GUS expression showed GUS staining. The rol B promoter was active in the columella of the cap, the initial zone, and the provascular meristem of the tip of the primordia. The results show that the rol B gene product enhances the rhizogenic potentialities of thin layers.

IN VITRO ROOTING COMPETENCE OF BASAL SPROUTS AND CROWN BRANCHES OF MATURE CHESTNUT*

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In vitro shoot cultures were established from basal sprouts (material retaining juvenile characteristics) and crown branches collected from the same mature (80-year-old) chestnut tree (*Castanea sativa* Mill.). Morphogenetic competence of both types of cultures were evaluated during the *in vitro* establishment, multiplication and rooting phases. Basal sprout-derived cultures exhibited a higher percentage of establishment, multiplication coefficient and rooting rates than cultures from crown origin.

To explain the morphogenetic characteristics recorded, some biochemical parameters were evaluated: proanthocyanindin content, endogenous levels of auxins and cytokinins, and peroxidase activity and soluble proteins. Basal shoot cultures contained higher amounts of proanthocyanidins and auxins and less cytokinins than crown cultures. The auxin/cytokinin ratio is always higher in rooting cultures (basal sprout origin). On the other hand, two protein bands have been observed in extracts from crown derived cultures which are not present in basal sprout cultures. These proteins could be taken as markers to define the degree of maturity within the different parts of the same tree.

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FACTORS AFFECTING ROOTING SUCCESS OF JACK PINE CUTTINGS: TREE AGE AND CROWN SAMPLING LOCATION

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Rooting success as indicated by percentage rooting, root length and number of roots, as well as new shoot length and cutting vigour ratings was determined in 5 different age classes of Jack pine (*Pinus banksiana* Lamb.). Different crown locations within the trees were sampled prior to bud burst (April 15, 1992) to investigate crown architectural influences. Cuttings from older trees (9-14 yrs) rooted poorly, ranging from 0-26%. Success was higher with lateral shoots from lower crown regions. Rooting percentages were higher (50.0-66.7%) for 3- or 5-year-old plants regardless of crown sampling location. Root length was generally greater for cuttings taken from lower crown regions and increased with decreasing age. There were no differences among numbers of roots, new shoot growth, and vigour of the cuttings.

FACTORS AFFECTING ROOTING SUCCESS OF JACK PINE CUTTINGS: PROPAGATION ENVIRONMENT, FERTILITY AND AUXIN APPLICATION

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In a series of rooting experiments on young (3-month-old) Jack pine (*Pinus banksiana* Lamb.) seedlings, effects of different rooting conditions, timing of nitrogen applications to donor plants and auxin delivery were investigated. Post rooting cutting survival was also studied. Rooting was higher with main axis cuttings as compared with first order laterals. In natural light (no shading), rooting was higher than when shade cloth (50%) was used to screen seedlings. Rooting percentage in peat:vermiculite (1:1 vol.) medium was higher than sand:perlite (1:1 vol.) only under shaded conditions. Low frequency (monthly) applications of fertilizer (150 ppm N:60 ppm P:150 ppm K) to donor plants resulted in higher rooting than either controls (no fertilizer) or high frequency (weekly) treatments. Auxin treatment (NAA, 10 sec., 1,000 ppm dip) increased numbers of roots observed but had no effect on overall percentage rooting success. Similar rooting was observed with water, ethanol or DMSO as carriers of NAA. Post rooting survival in general was high. Rooted cuttings classified into medium and high groupings, based on root length, numbers of roots and root branching, survived greater than 80% in a variety of environmental conditions.

ROOTING *IN VITRO* OF MICROPROPAGATED SHOOTS OF *PRUNUS AVIUM*, *P. PADUS* AND *FRAXINUS EXCELSIOR* IS PROMOTED BY PHLOROGLUCINOL

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During the first 500 days of monthly subculture, phloroglucinol (1,3,5 trihydroxybenzene; PG) at 1 mM increased the proportion of micropropagated shoots that rooted with auxin from several mature genotypes of *P. avium* (wild cherry), but later the shoots apparently became rejuvenated since they were able to root with auxin alone. PG alone was able to induce rooting, and the response to PG was apparently unrelated to the degree of rejuvenation of the culture. Prior to rejuvenation of the shoot cultures, PG was more effective than auxin in inducing root formation. Increasing the concentration of PG above 1 mM in medium without auxin resulted in a corresponding increase in the proportion of shoots that rooted and numbers of roots per shoot. In medium with auxin, only root numbers were affected by PG concentration. PG also increased the proportion of *P. padus* (bird cherry) shoots that rooted with auxin.

Since most previous studies with PG involved members of the Rosaceae, the effects of PG upon root formation by micropropagated shoots of *F. excelsior* (common ash; Oleaceae) were investigated. The response to auxin of shoots from cultures derived both from seedlings and from a mature tree, varied considerably. However, in most cases, poor rooting with auxin could be compensated for by the addition of PG.

SEASONAL VARIATION IN THE ROOTING ABILITY OF *SALIX PLANIFOLIA* AND *POPULUS BALSAMIFERA* IN SUBARCTIC QUÉBEC

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The rooting ability of cuttings from five populations of *Salix planifolia* and five clones of *Populus balsamifera* growing along the Great Whale River in subarctic Québec was determined. Each population and clone was sampled monthly, from May to October in 1992 (six sampling episodes). There were significant differences among clones of *Populus*, but not among populations of *Salix* in cutting rooting potential. Cuttings of *Populus* from recently burned clones rooted more profusely than 'older' clones. For both species, cuttings from earlier (i.e., May) and later (i.e., October) in the season produced more roots, and these were on average longer than those collected in the middle of the growing season (i.e., July). Differences in the physiological characteristics of dormant and actively growing individuals appear to be of significance in determining the rooting ability of cuttings for both species studied.

THE ROOTING POTENTIAL OF *JUNIPERUS COMMUNIS* CUTTINGS FROM FIVE SUBARCTIC POPULATIONS

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We determined the rooting potential of *Juniperus communis* cuttings from five dune populations ordered along a south-north latitudinal gradient on the eastern coast of the Hudson Bay. The populations occupied the transition zone between the northern part of the boreal forest and the forest tundra. Male and female individuals from each population were sampled and cuttings were treated with either 8,000 or 12,000 ppm of a rooting hormone (IBA). There was no clear latitudinal gradient in the rooting potential of *Juniperus* cuttings, although those from southern populations tended to form fewer roots than those from northern populations. Cuttings from female plants produced significantly more roots than those from male plants. Cuttings from male individuals did not respond to the rooting hormone levels, but cuttings from female individuals produced fewer roots at the higher IBA concentration. Root length was not significantly affected by cutting origin, plant gender, nor hormone concentration. The physiological basis for greater rooting ability of cuttings from female plants is not clear, but may be related to hormonal balance within the plant.

PROMOTED ROOTING OF *SYRINGA VULGARIS* 'ALBA' CUTTINGS

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Rooting of *Syringa vulgaris* 'Alba' softwood tip cuttings was studied after stockplant etiolation, banding the proximal 3 cm of the future base of cuttings with aluminium foil and/or treating the cuttings with 3,000 ppm indolebutyric acid (IBA) after 1 or 2 weeks of greening period. The interaction of all the treatments were also tested.

The effect of etiolation was most drastic: it increased the mean number of roots 2-3 times as compared to the similarly treated non-etiolated cuttings. Banding did not affect root number. A longer greening period decreased and IBA treatment slightly enhanced root initiation. No interactions between any of the treatments were found to affect root number. A larger proximal region was involved in root initiation if the cuttings had been etiolated or banded, the region being largest if both of these treatments were applied. The amount of callus tissue on the cutting bases was highly significantly affected by treatments and higher root numbers were related to lower amounts of callus. Browning and death of the cutting apices were decreased by 75% if cuttings had been etiolated.

THE ROLE OF SCLEREIDS IN DETERMINING ROOTING POTENTIAL IN STEM CUTTINGS OF *CARPINUS BETULUS* 'FASTIGIATA'

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Etiolated, shaded and light-grown shoots of *Carpinus betulus* 'fastigiata' were sampled over a 16-week period following treatment for studies of rooting and stem anatomy. Rooting declined with cutting age, and stock plant etiolation or shading dramatically increased rooting potential up to 12 weeks after treatment. Numerous anatomical changes were correlated with stem age and stockplant treatment. Etiolation reduced the lignification of the secondary xylem and the thickness of the periderm, and increased the percentage of sclereid-free gaps in the perivascular sclerenchyma. Shading also reduced the total number of sclereids and increased the percentage of sclereid-free gaps. Rooting capacity was positively correlated with changes reflecting increased stem succulence, i.e., wider pith and cortex, thinner periderm, less lignification of the secondary xylem, and a higher percentage of sclereid-free gaps in the sclerenchymatic sheath. Stem succulence was greatest in both young and etiolated or shaded stem cuttings. It is proposed that while schlerenchymatic sheaths do not present physical barriers to root initiation in most stem cuttings, the development of sclereids does reduce rooting potential by usurping potential root initiation sites. In essence, non-living, lignified sclereids are unable to de-differentiate to root initials or to be included in developing root primordia. Excluding light from developing shoots retarded sclereid development by up to 3 months following treatment. This correlated well with observed increases in the rooting potential of etiolated or shaded stems.

STIMULATING BUD BREAK IN ETIOLATED AND NON-ETIOLATED ROOTED SOFTWOOD CUTTINGS

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The objectives of this research were to stimulate new shoot growth in newly rooted cuttings of difficult-to-root woody plants thereby accelerating growth in the propagation year and increasing overwinter survival rates. Etiolated and non-etiolated cuttings were taken from field grown stock plants of *Acer rubrum*, *Hamamelis vernalis*, *Hamamelis virginiana*, and *Stewartia pseudocamellia*. After a 4 week etiolation period, during which the bases of new shoots were covered in black velcro bands, cuttings were taken in June, 1992 and dipped in IBA at three levels; 500, 1,000, and 5,000 ppm for *Acer rubrum* 'October Glory', *Acer rubrum* 'Red Sunset', and *Hamamelis vernalis*, and 1,000, 5,000, and 10,000 ppm for *Hamamelis virginiana* and *Stewartia pseudocamellia*. After 3 to 8 weeks under mist, successfully rooted plants were potted into a 1:2:1, by volume, soil-peat-perlite medium amended with dolomitic limestone 103 g/bu, ground gypsum 41 g/bu, 20% super phosphate 41 g/bu, Micromax (31 g/bu), $CaNO_3$ (10 g/bu), and KNO_3 (10 g/bu). Plants were given 16 hour extended days with 70/60°F day/night greenhouse temperatures. Plants were treated with foliar sprays of thidiazuron (50 mg/L), thidiazuron followed by $GA_{4/7}$ (250 mg/L) 10 days later, silver thiosulfate (STS) (1%), or STS followed by $GA_{4/7}$ 10 days later. Both STS and thidiazuron stimulated bud break in plants. After 4 weeks of long days and 70/60°F day/night temperatures, plants were assessed for bud break, after which dormancy was induced under short days and cool temperatures (50/40°F day/night). Once dormant, plants were assessed for total growth and stored carbohydrate levels and stored in 38°F coolers for 3 months. Plants were then removed from cold storage and assessed for overwinter survival.

ADVENTITIOUS ROOT FORMATION—A CRITICAL STAGE IN DISTURBANCE-INITIATED CLONAL REGENERATION OF PACIFIC YEW (*TAXUS BREVIFOLIA*)

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Studies on the Ashland Ranger District of the Rogue River National Forest in the high Cascade subprovince of southwestern Oregon contribute to the identification of environmental and biotic factors that influence clonal reproductive strategies in Pacific yew. Because taxads lack specialized vegetative structures to initiate clonal growth, they depend upon external mechanisms to express their clonal potential. Analysis of post-disturbance events in *Taxus* species calls attention to initiation of adventitious roots as an early response in clonal regeneration. Following natural or anthropogenic disturbance events, fallen *Taxus* stems respond to contact with forest litter. Under these conditions, soil moisture condensing at the bole-litter interface promotes production of adventitious roots. Development of functional root systems is critical for the establishment of independent ramets.

A HISTOLOGICAL STUDY OF ADVENTITIOUS ROOT FORMATION IN TWO MORPHOLOGICAL FORMS OF STEM CUTTING FROM *EUCALYPTUS GRANDIS*

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Differences in rooting ability were observed in cuttings prepared from *Eucalyptus grandis* shoots with different cross-sectional morphologies. Cuttings with a rectangular stem cross-section rooted more quickly and more prolifically than cuttings with a circular cross-section. Transverse sections of stem tissue revealed no gross anatomical differences in tissue organisation between the two stem morphologies. Counts of developing adventitious root meristems confirmed that root development occurred more frequently and earlier in rectangular stems than in circular stems. Stems of both morphologies possess a rectangular pith. Adventitious root meristems developed in the vascular cambium, most frequently centrifugal to one of the corners of the rectangular pith. These zones are associated with the vascular tissues of the leaf traces. Development of secondary xylem in the cuttings after setting in the propagation bed appeared to be a prerequisite for the formation of adventitious root meristems. There was an inverse relationship between the width of secondary xylem in the stem of the cutting sampled, and number of adventitious root meristems observed. Adventitious root meristems were found in rectangular section stem tissues taken from within 7.5 mm of the base of cuttings and from a height of 7.5 - 15 mm above the base of the cuttings. Fewer root meristems were found in circular section tissues, but the majority of those meristems observed were found within 7.5 mm of the base of the cutting. Selection of shoots with rectangular morphology ensures that cuttings set for rooting have the potential to root rapidly and produce a vigorous, well distributed root system.

INDUCTION OF ROOTING

POSTER ABSTRACTS

EFFECT OF NAA AND SALT CONCENTRATIONS ON ADVENTITIOUS ROOT FORMATION OF *IN VITRO* SHOOTS IN *ACACIA MEARNSII*

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Shoots produced *in vitro* are usually transferred to another medium for adventitious root formation. Although in some species shoots are rooted on a hormone-free medium, most rooting media contain a suitable level of an auxin and often have reduced basal salt concentrations. This study was conducted to define a suitable medium to stimulate rooting in *in vitro*-produced shoots of black wattle (*Acacia mearnsii* Wilde), the world's principal source of tannin. Our objectives were to: (1) evaluate the effect of ∝-naphthaleneacetic acid (NAA) concentration on root formation, and (2) observe the rooting response to salt concentrations. Shoots used in this study were maintained *in vitro* on Murashige and Skoog (MS) medium containing 2 mg/L benzylaminopurine (BAP) and 0.01 mg/L indole-3-butyric acid (IBA). Shoots 2- to 3-cm long were transferred to rooting medium and maintained at 25 ± 5°C exposed to 12-hour photoperiods of cool-white fluorescent light (70 $\mu Em^{-2} s^{-1}$). Rooting medium consisted of either full- or half-strength MS medium supplemented with 20 g/L sucrose, 6 g/L agar, and NAA at 0, 0.2, 0.4, 0.6, or 0.8 mg/L. The percentage of rooting and the number of roots produced per shoot were higher on half-strength than on full-strength MS medium. As the concentration of NAA increased, percentage of rooting increased reaching a maximum with 0.6 mg/L. Half-strength MS with 0.6 mg/L NAA appears suitable for *in vitro* root induction in *A. mearnsii*.

CHANGES IN CARBOHYDRATE CONTENT DURING RHIZOGENESIS IN *VITIS* CUTTINGS STORED AT 2°C

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Carbohydrates play an important role in the morphogenetic processes and their amount and quality differ during the vegetative cycle. By treating cuttings of *Vitis* rootstock 140 Ruggeri with cold and dipping in water, we changed the timing of rooting and at different stages of the rooting process we analyzed the sucrose, glucose, and fructose compositions of the cuttings.

The results showed that: (1) for the cold-treated cuttings the values at time 0 are 60% lower when compared with those collected in the field, showing that the cold treatment precociously mobilizes carbohydrate reserves, thus enhancing the rooting process; (2) the cuttings during the first 20 days lost 30% of glucose, fructose, and sucrose; (3) the cuttings collected in December (dormancy period) utilized the carbohydrates more slowly; and (4) under our experimental conditions, dipping in water did not change the above mentioned pattern.

BACTERIAL STIMULATION OF ADVENTIVE ROOT FORMATION IN PINE

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We have isolated and cultured a bacterium that, when co-cultured under *in vitro* conditions with *in vitro* produced microshoots of axillary origin or seedling-produced hypocotylary cuttings, initiates adventitious rooting. The efficiency of this initiation process ranges from 40 to 90% depending upon the physiological condition, chronological age and morphogenetic source of the explant. The process seems not to be species specific as several *Pinus* species have responded positively to the root inducing bacterial treatment. Under all conditions studied to date, the organism has proven not to be pathological to the target tissue. The organism has been used for the induction of adventive rooting under *in vitro* conditions in both slash pine (*Pinus elliottii*, Englm.) and white pine (*Pinus strobus* L.). Experiments have been initiated to determine rooting efficacy under *ex vitro* conditions under conventional greenhouse culture conditions.

EFFECT OF GIBBERELLIC ACID ON ALMOND ROOTING

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The effect of gibberellic acid on adventitious root formation is not completely known. Several contradictory results have been obtained: GA_3 inhibits root formation in some species but improves rooting in others. Some authors suggest that GA_3 effects could be explained by an action on the activity of the peroxidases which play an important role in auxin metabolism. The aim of this work was to evaluate the effect of gibberellic acid on rooting of almond (sel.MN51) and the importance of its action on peroxidase activity related to the rooting. Gibberellic acid was used either during multiplication to prepare the explants for rooting or added to the rooting medium. During rooting GA_3 was used at 3 different concentrations for short periods at different times during the first days of rooting in order to influence peroxidase activity and improve rooting.

GA_3 used as a pretreatment in the multiplication phase did not improve rooting ability. GA_3 used in the rooting medium lowered peroxidase activity and showed variable effects in relation to the period of treatment and the dosage.

THE INFLUENCE OF CYTOKININ AND AUXIN ON THE ROOTING CAPACITY OF TRANSGENIC TOBACCO LEAF EXPLANTS EXPRESSING SINGLE *ROL* GENES OF *AGROBACTERIUM RHIZOGENES*

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The formation of adventitious roots in leaf explants from wild type and transgenic tobacco expressing either *rol A*, *rol B* or *rol C* genes was investigated. The explants were cultivated on MS medium with or without exogenous cytokinins (6-benzylaminopurine or kinetin) and auxin (NAA, 1-naphthaleneacetic acid), and the endogenous content of cytokinins in explants was measured during the period of root formation by means of an ELISA method. Rooting was poor without auxin treatment. The wild type, *rol A* and *rol C* formed hardly any roots and *rol B* rooted only slowly. A considerable stimulation of rooting was given by treatment with 1μM NAA in *rol B* explants which formed numerous roots already after 4 days cultivation. *Rol A* was stimulated to a lesser extent by this treatment whereas the wild type and *rol C* responded only weakly to the NAA treatment. The optimal concentration of NAA for rooting was 1.0 μM and other concentrations in the range 0.001-10 μM resulted in no or less stimulation. Cytokinins inhibited rooting in the wild type, *rol A* and *rol C* explants, although this inhibition was not complete in *rol B*. The inhibitory effect of 0.001-1.0 μM kinetin on rooting in *rol B* could only be reversed to a limited extent by simultaneous treatment with 1.0 μM NAA. It is known that the *rol B* gene increases the sensitivity of transgenic tissue to exogenous auxin. This is possibly the main reason why *rol B* leaf explants rooted so fast and abundantly in response to the NAA treatment. The results also indicate that this high sensitivity to exogenous auxin is connected with an unusually narrow optimal concentration range of NAA for rooting as 0.1 and 10.0 μM did not support rooting effectively. How cytokinin counteracts the effects of auxin in rooting is not known, and whether cytokinin influences the sensitivity of transgenic tissue to exogenous auxin *in vitro* needs further investigation. Preliminary cytokinin measurements indicated that the content of endogenous cytokinins increased more slowly in the *rol B* explants than in other materials. The results from these measurements will be discussed in relationship to the rooting ability studies.

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CULTURE MEDIA AND PLANT FACTORS INFLUENCING THE ROOTING OF NODAL EXPLANTS OF *EUCALYPTUS SIDEROXYLON IN VITRO*

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Nodal explants excised from *Eucalyptus sideroxylon* seedlings were utilized for *in vitro* studies of root initiation and elongation. Modified half-strength Murashige and Skoog (MS) medium supplemented with different levels of sucrose and auxin were tested to identify optimal combinations for rooting and plantlet formation. The effect of activated charcoal on root growth under various conditions was determined. Explants from distal or proximal segments of the mainstem also were tested for rooting capacity and subsequent pattern of plantlet growth and performance. Root development was favored by 2-6% sucrose concentrations in the medium. Higher percentages of sucrose caused callusing at the base of explants. A rooting medium with a combination of 2.5 μM indolebutyric acid (IBA) and 2.5 μM a-naphthaleneacetic acid (NAA) was most suitable for rooting and plantlet development. Addition of activated charcoal to a medium with auxin favored elongation of roots initiated *in vitro* by stimulating cell elongation but inhibited root initiation and subsequent root number per explant. Explants from well-rooted donor plantlets exhibited less basal callus, a high rooting frequency and more well-developed root systems in subsequent subcultures. These results suggest that root and basal callus formation are influenced by hormone and sucrose concentrations of the culture medium as well as the physiological and biochemical status of individual plants.

IDENTIFICATION OF ROOT PROMOTING SUBSTANCES IN A COMMERCIAL SEAWEED CONCENTRATE

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Using the mung bean rooting bioassay, evidence was found for the presence of heat stable, translocatable root promoters in a commercial seaweed concentrate prepared from the brown alga *Ecklonia maxima* (Osbeck) Papenfuss. The optimum response was obtained with a 10% solution. This concentration also promoted the rooting of six species of garden plants.

Compounds active in the mung bean rooting bioassay were isolated from the neutral indole fraction of the seaweed concentrate by high performance liquid chromatography. A gas-chromatographic-mass spectrometric analysis of the most active fractions indicated the presence of the following indoles: indole-3-acetic acid; indole-3-carboxylic acid; *N,N*-dimethyltryptamine; indole-3-aldehyde; and in addition, *iso*-indole, 1,3-dione (*N*-hydroxyethylphthalimide). This appears to be the first report of an indole amine and a phthalimide in algae. Attempts are currently being made to determine the efficacy of these compounds in adventitious root formation.

EFFECTS OF PUTRESCINE AND INHIBITORS OF PUTRESCINE BIOSYNTHESIS ON ORGANOGENESIS IN *EUPHORBIA ESULA* L.

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The inhibition of root formation by simultaneous application of difluoromethylornithine (DFMO) and difluoromethylarginine (DFMA), two specific suicide inhibitors of putrescine biosynthesis, was reversed by IAA or IBA when they were applied to aseptically isolated hypocotyl segments of leafy spurge (*Euphorbia esula* L.) grown in darkness on agar medium containing B5 salts and vitamins. Putrescine (0.5 mM or higher concentrations) inhibited root formation when the B5 salts and vitamins were diluted to 10% or 30% of their normal concentrations, but it did not inhibit root formation in the normal B5 medium. In the 10% medium, 0.25 mM putrescine did not inhibit root formation, nor did it reverse the inhibition of rooting due to DFMA + DFMO treatment. However, IAA and IBA did reverse the inhibition by DFMA + DFMO in the 10% medium. An inhibitor of spermidine biosynthesis, methyl glyoxal-*bis*-guanylhydrazone, also inhibited root formation in hypocotyls grown in the 10% medium; but, in this case, IAA did not reverse the inhibition. This suggests that putrescine is not required for auxin-induced root formation in this system, but spermidine or spermine may be required.

PHASES DURING ROOT FORMATION IN MICROCUTTINGS OF APPLE

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Rooting can be divided into several successive phases from which differentiation (i.e., the formation of an adventitious root-meristemoid) is the most important. We assumed that BAP blocks rooting most effectively when added during this phase. Short 24 hour pulses with BAP strongly inhibited rooting when given between 24 hours and 96 hours after transfer to rooting medium. Thus, differentiation occurred during this period. In agreement with this, a 24 hour pulse of auxin was most effective when given between 24 and 96 hours. The lag period up to 24 hours may represent a phase of dedifferentiation. After 96 hours, the outgrowth phase occurred. Microscopic analysis showed that meristemoids were present after 48 hours. A BAP pulse at that time resulted in unorganized cell divisions.

We currently examine the effect of growth substances during the various periods. Pulses with 2,4-D showed that this auxin was less effective in inducing rooting than IBA, but more effective in inhibiting root outgrowth. Pulses with phloroglucinol showed that this phenolic compound is unlikely to have acted in the same period as auxin. This casts doubt on its supposed role as an auxin-protectant.

SITE-SPECIFIC POLYMORPHIC TUMORIGENESIS INDUCED IN SINGLE *LARIX DECIDUA* GERMLINGS BY *AGROBACTERIUM RHIZOGENES*

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Larix decidua germlings showed any one or collectively all of apical teratomas, bud clusters, shooty bud tumors, and root tumors depending upon the site(s) of host wound-inoculation with *Agrobacterium rhizogenes* pRill325. Inoculation of only a very shallow wound through the center of the shoot apex resulted in a large and confluent teratoma of bud needles and bud primordia which failed to develop further. A more profound (1 - 2 mm) through the apex generated varying numbers of elongating shoots and/or clusters of static buds from tissues immediately peripheral to the wound. Inoculation with pRill325 made to wounds in the lower hypocotyl induced only root tumors. Inoculation with *Agrobacterium rhizogenes A4pARC8* made to apical or hypocotyl wounds evolved only root tumors. Frequency of tumorigenesis by either pRill325 or A4pARC8 was virtually 100%. Transformation was verified by Southern blot analyses.

INTERACTION OF INDUCTIVE AND INHIBITORY GROWTH REGULATOR BALANCES ON ROOT ORGANOGENESIS

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In tobacco leaf explants cultured on MS medium, the bundle sheath and vein parenchyma cells can be induced to divide to form root primordia by treatment with 1μM IBA. These roots are formed directly and no intervening callus phase occurs. The cells are competent for root induction from the time of excision and only a short exposure period to IBA of around 1-2 days is required for subsequent root formation, although longer exposure of up to 10 days does lead to larger numbers of roots being produced. Normally, no roots are formed on basal medium. However, root induction can be disrupted such that virtually no roots are produced if 1μM BAP is included in the medium along with IBA. Removal of the BAP by transfer of the explants at intervals to medium with only IBA releases the inhibition and allows root induction to proceed. Transfer of explants from an inductive to inhibitory medium and *vice verse* reveals that inhibitory and inductive growth regulator balances act at a specific time during the organogenic process. A period of 1 or 2 days after determination appears highly sensitive to the growth regulator balance in the medium. This is at the point when the bundle sheath cells are beginning their first division. During this time BAP can inhibit root formation suggesting that the explant is only weakly canalised for root formation during this period.

ADVENTITIOUS ROOT FORMATION IN MICROCUTTINGS OF EASTERN REDBUD (*CERCIS CANADENSIS*)

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Adventitious root formation was evaluated in four mature clones and one juvenile clone of eastern redbud. All clones were successfully rooted following a 15 day pulse treatment *in vitro* on half-strength WPM containing 150 or 300 µM IBA. Subsequent root development proceeded *ex vitro* in cell packs in a peat:perlite medium under high humidity. Differences were observed between clones with rooting frequency varying from 53 to 93% and mean roots per cutting between 2.9 and 5.5. The number of roots initiated per cutting was strongly related to the size of the microcutting. Cuttings greater than 4 cm initiated the highest number of roots per cutting. Anatomical observations showed root primordia formation by 15 days on the auxin medium. In the juvenile microcuttings, roots formed directly from cells in the phloem parenchyma. In contrast, root primordia organized in mature microcuttings indirectly within files of cells originating from the phloem parenchyma. Adventitious roots from both juvenile and mature microcuttings had strong vascular connections with the parent cutting after 25 days.

ANALYSIS OF ADVENTITIOUS ROOT INITIATION IN EASTERN WHITE PINE CUTTINGS

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We are studying control of adventitious root initiation in eastern white pine (*Pinus strobus*). We have developed an experimental system in which approximately 40 root initials form on 2-week-old hypocotyl cuttings within 4 weeks following a 5-minute pulse treatment with 300 mg/l NAA. Histological examination of the 2-week-old hypocotyls and of epicotyl cuttings from 11-week-old seedlings showed that NAA treatment speeded root initiation in cuttings from both ages. In addition, cuttings from the 11-week-old seedlings initiated roots more slowly than those from the younger seedlings. The first organized cell divisions leading to root primordium formation were observed 4 days after NAA treatment in the 2-week-old cuttings, but much later in the other treatments. We also observed differences in the site of origin of adventitious roots depending on NAA treatment and seedling age. Control cuttings from 11-week-old seedlings initiated roots from wound tissue formed at the base of the cutting, whereas cuttings from the other three treatments initiated roots from cells external to vascular tissue and adjacent to resin ducts.

We are using this system to investigate the role of gene expression in root initiation. We have prepared cDNA libraries from mRNAs of NAA-treated and control hypocotyl cuttings 4 days after pulsing. Because only the NAA-treated hypocotyls have begun the root initiation process at that time, identifying cDNAs expressed differentially in the two libraries should allow us to isolate root initiation-specific genes. Putative rooting genes will also be screened against mRNAs from NAA-treated cuttings of 11-week-old seedlings to eliminate genes expressed in response to auxin treatment, but not specifically associated with rooting. We plan to use genes expressed early in the root initiation process as markers to study the control of adventitious root initiation and changes in rooting competence with plant age.

EFFECT OF NAA, BA, AND SALT CONCENTRATIONS ON *IN VITRO* ADVENTITIOUS ROOT PROLIFERATION OF ASCLEPIAS TUBEROSA

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Butterfly flower (*Asclepias tuberosa* L.), an herbaceous perennial, has potential as a flowering pot plant and cut flower. *In vitro* multiplication could provide a means of capturing desirable phenotypes such as color variants and growth habits. Therefore, research was conducted to study *in vitro* root formation in this species. Our objective was to evaluate responsiveness of adventitious root formation and proliferation to ∝-naphthaleneacetic acid (NAA), benzylaminopurine (BA) and basal salts. Shoot tips 5 mm long were collected from 3-week old greenhouse-grown seedlings and cultured on Murashige and Skoog minimal organic (MSMO) medium, either full-strength or half-strength, supplemented with 30 g/L sucrose, and 8 g/L agar. Plant growth regulator treatments were a factorial of BA (0, 1, 2.5, 5, or 10 mg/L) and NAA (0, 0.01, 0.1, 0.5, or 1 mg/L). The cultures were maintained under 10-hour photoperiods of cool-white fluorescent light (50μ Em^{-2} s^{-1}) at 21 ± 3°C. Results showed that the number of roots and percentage of rooting was greater at higher concentrations of NAA. Also, more root proliferation occurred on half-strength than on full-strength medium. Maximum root length was observed with 0.5 mg/L NAA. These results will be useful in developing a micropropagation system for Butterfly flower.

SCREENING FOR ROOT INDUCTION GENES AND TRANSFORMATION IN *PINUS CONTORTA*

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Our system to find rooting genes in conifers is based on the assumption that side (lateral) root formation is fundamentally similar to rooting of cuttings. We have prepared root pieces from near the apex and from the basal part of the root from *Pinus contorta* seedlings. From these pieces RNA was isolated and two cDNA libraries were made from the mRNA. Phage λ-zap was used as the vector for both cDNA libraries and these are currently being screened for differences. Hypocotyl cuttings treated with auxin to induce rooting (see poster by Flygh and Grönroos) will be used to test putative rooting-related cDNA clones by *in situ* hybridization.

Transformation of *Pinus contorta* hypocotyls with *Agrobacterium rhizogenes* resulted in transformed roots expressing ß-glucuronidase (the GUS gene) and opines. This technique will be of interest when studying isolated root induction genes in intact roots.

T-DNA *ROL* GENES: AN APPROACH TO IMPROVE RHIZOGENIC ABILITY IN WOODY SPECIES

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Root formation at the inoculation site is the typical response of the dicot species to *Agrobacterium rhizogenes*. T-DNA *rol* genes are known to affect adventitious rooting and, among them, ORF 11 (*rol* B) has been found to be the gene responsible of this morphogenetic event in the hairy root pathogenesis. The function of *rol* B has been elucidated as well as those of other *rol* genes. Generally, the activities of these bacterial genes (from T-DNAs of both *A. rhizogenes* and *A. tumefaciens*) are involved, even if with different mechanisms, in the hormonal metabolism of the plant cell.

By transferring these genes into plants, it is possible either to have information on the root induction mechanism or to induce/improve rooting ability in woody species, whose propagation by cutting is strongly limited by the achievement of an adequate root apparatus.

ANATOMY OF ADVENTITIOUS ROOT FORMATION IN MICROCUTTINGS OF *MALUS DOMESTICA*

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Anatomical events of adventitious root formation during root induction and post-induction treatment were observed in microcuttings of *Malus domestica* Gala. Treatment with root induction medium containing water, 1.5 μM 1H-indole-3-butyric acid (IBA), 44 mM sucrose, or 1.5 μM IBA + 44 mM sucrose for 4 days averaged in 0, 0.2, 2.2, and 11.9 meristemoids per microcutting, respectively. Meristemoids in sucrose treatment were associated with leaf gaps and traces. Time course analysis of root induction treatment with 1.5 μM IBA + 44 mM sucrose over 4 days showed some phloem parenchyma cells were densely cytoplasmic with nuclei having prominent nucleoli in 1 day; phloem meristematic activity was extensive by 2 days; dividing phloem parenchyma cells progressed into the cortex by 3 days; and identifiable root primordia were present by 4 days. Primordia were restricted to the basal 1 mm of microcuttings. Cell division occurring in pith, cambial region, and cortex did not lead to primordia formation. Post-induction treatment time course analysis showed distinct differentiation at distal ends of primordia in 1 day; conical shaped primordia with several distal cell layers by 2 to 3 days; roots having established tissue systems emerging from the stem in 4 days; and by 6 days numerous roots had emerged. Results showed root initiation was detectable in 24 hours, completed in 4 days of induction treatment, and involved phloem parenchyma cells.

THE ROLE OF pH IN 1H-INDOLE-3-BUTYRIC ACID INDUCED ROOT INITIATION IN MICROCUTTINGS OF *MALUS DOMESTICA*

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Involvement of pH in auxin induced adventitious root initiation was investigated with microcuttings of *Malus domestica*. Root initiation was induced aseptically on 5-node apical microcuttings using 44 mM sucrose for 4 days in dark at 30°C and 4-day applications of 1H-indole-3-butyric acid. Unbuffered initiation medium pH rose from 5.6 to 7.0 within 2 days of microcutting incubation. Including 10 mM 2[*N*-morpholino] ethanesulfonic acid (MES) buffer adjusted to pH 5.5 with KOH, NaOH or CaOH in medium prevented pH change and resulted in 14.3, 14.5 and 6.3 roots per microcutting, respectively, compared to 7.8 roots in unbuffered medium. Microcuttings buffered with 10 mM MES/KOH at pH 5.5 produced 2 fold higher root numbers (14.2 roots) than ones at pH 7.0 (7.1 roots). Influence of pH on root initiation depended on IBA concentration. Root numbers produced in 1.5 µM IBA or less followed a trend of pH 5.5 > 6.25 > 7.0 while at 15 µM IBA the trend was pH 6.25 > 5.5 > 7.0 and at 150 µM IBA pH had no affect. The cultivars Gala, Jonathan, Triple Red Delicious, and Vermont Spur Delicious responded to pH similarly with higher root numbers and higher uptake of IBA at pH 5.5 than at 7.0. Higher root numbers at lower pH are thought to be due in part to greater IBA uptake.

CHANGES IN PEROXIDASE ACTIVITY, ENDOGENOUS AUXIN LEVEL AND ETHYLENE PRODUCTION DURING THE COURSE OF ROOT FORMATION BY POPLAR SHOOTS RAISED *IN VITRO*

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Shoots of poplar (*Populus tremula* x *P. tremuloides*) were multiplied *in vitro* and rooted on a rooting medium in the presence of NAA. No rooting occurred in the absence of exogenous auxin. A peak of soluble peroxidase activity specifically preceded rooting as generally observed in other materials by other authors. This peak corresponded to a lowering of the free IAA level in the shoots. These events were considered as corresponding to the initiative phase of rooting. They are preceded by a peaking of the free IAA level which might achieve the inductive phase of the rooting process. A burst of ethylene production was measured both in rooting and in non-rooting shoots, but the ethylene peak from rooting shoots appeared earlier and was higher. The use of ACC indicates that the exogenous auxin might have enhanced ACC-synthetase activity. The precocious ethylene peak appears as the first biochemical event associated to the rooting process.

PRACTICAL USES OF PEROXIDASE ACTIVITY AS A PREDICTIVE MARKER OF ROOTING PERFORMANCE OF MICROPROPAGATED SHOOTS

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A peak of specific peroxidase activity (an increase of enzyme activity followed by a decrease) has been generally found in crude extracts of stem or shoot cuttings prior to root formation. Effectors, some of them of a phenolic nature, play a role in these changes of peroxidase activity. This may explain discrepancies in some investigations dealing with partially purified extracts or cell fractions. The peroxidase peak does not terminate the rooting inductive period as proposed some years ago, but rather the initiative phase before identification and growth of endogenous root primordia. Depending upon the materials studied, characteristics of the peroxidase peak such as precocity of appearance, height, and velocity of decrease have been correlated with ultimate performance of rooting. Physical and/or chemical factors which were able to modulate the peak, also had effects on the subsequent rooting rate. An early test using peroxidase activity of extracts made from shoots before transferring to rooting media discriminated in favour of the phenol compounds capable of enhancing the rooting process and determined the moment of application in relation to the peroxidase peak.

CHARACTERIZATION OF AN *ARABIDOPSIS THALIANA* MUTANT SELECTED FOR ENHANCED ABILITY TO FORM ADVENTITIOUS ROOTS

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Initiation of adventitious roots is one of many auxin-induced developmental processes. The degree to which an auxin stimulus is manifested as root initiation depends on tissue-specific sensitivity to auxin and the concentration of auxin at receptive sites. Auxin concentration is regulated by biosynthesis, transport, compartmentation, and metabolism. To examine genetic factors contributing to variation in rooting responses, an EMS mutagenized M_2 population of *Arabidopsis thaliana* was screened for mutants with altered abilities to form adventitious roots. A selected recessive nuclear mutant, *rooty* (*rty*), is characterized by overproduction of adventitious and lateral roots and inhibition of shoot development. A phenocopy of *rty* occurred when wild type seedlings were grown on medium containing 100 µM IBA. Normalized growth of *rty* was exhibited on media containing the cytokinin 2iP at 12 and 24 µM. Gravitropism in 6 day old dark grown seedlings was identical in wild type roots and shoots and in *rty* roots (3.5° from horizontal/h over 24 hours). However, reorientation rate in *rty* shoots was 1.6°/h during the first 24 hours and only 40° in 96 hours. Root cultures of wild type required exogenous auxin to sustain growth, whereas *rty* root cultures were auxin autotrophic. Based on phenotypic data and preliminary biochemical analysis, it appears that homozygosity of the *rty* allele causes an increase of endogenous IAA. Further characterization of *rty* may enhance the understanding of mechanisms regulating auxin concentrations.

THE MODE OF ACTION OF IBA ON ROOT FORMATION ON THIN STEM SLICES OF MICROCUTTINGS OF *MALUS* IS PARTLY VIA CONVERSION INTO IAA

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We developed a test system for root formation *in vitro*. This system consists of thin stem slices (with a fresh weight of ca. 1 mg) of *in vitro* cultured apple (*cv.* M9 Jork) shootlets. Incubation during 3 days on medium supplemented with 3.2 μM of indolebutyric acid (IBA) resulted in maximal root formation (ca. 8 roots per slice). The mode of action of IBA on root formation was studied by relating the "active auxin concentration" in the tissue to adventitious root formation. "Active auxin" comprises the free IAA acid (IAAH) resulting from IBA conversion and possibly also the free IBA acid (IBAH). The amount of IBAH was 2.5% and of IAAH 0.6% of the total of IBA taken up from the medium. To assess whether or not IBA acts solely *via* conversion into IAA, we also incubated stem slices on medium with radioactive IAA. After uptake of IAA only 0.6% was extracted as the free acid. IAA was not converted into IBA. Since the uptake of IBA was four-fold higher than the uptake of IAA, an incubation on medium with IBA leads to a higher IAAH concentration in the tissue than an incubation on medium with IAA. Preliminary results from experiments in which the endogenous IAAH concentrations were measured after incubation of stem slices on medium with IBA or IAA (using an ELISA technique) support these findings. Incubation on medium with IBA and IAA leading to equimolar IAAH concentrations in the tissue resulted in the case of IBA in significantly more roots than in the case of IAA. (IAA induced a maximum of only 4.3 roots per slice). From these results we conclude that IBA acts partly *via* conversion into IAA. Currently we are isolating genes involved in the induction of root formation. The function of these genes will be studied during *in vitro* root formation.

COMPARISON OF ADVENTITIOUS ROOTING RESPONSE TO AUXIN IN WILDTYPE AND MONOGENIC, AUXIN-RESISTANT MUTANT STEM CUTTINGS OF TOBACCO

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The initiation and development of adventitious roots is a vital process to successful clonal propagation of cuttings. Some species or developmental phases within species produce adventitious roots readily from stem cuttings while others are difficult to root or will not root at all. The goal of this research is to understand the molecular basis for differences in rooting ability among stem cuttings. Here, we describe an *in vitro* model system for comparing gene expression following an auxin treatment that induces adventitious root initiation in cuttings of one genotype but not in another genotype of tobacco (*Nicotiana tabacum* v. Xanthii). Axillary shoots from wildtype tobacco can be induced to produce adventitious roots with an exogenous IBA treatment; axillary shoots from monogenic, auxin-resistant mutants of the wildtype will not produce adventitious roots given the same IBA treatment. Histological evidence of the response to auxin in the wildtype and mutant genotypes will be presented.

ROOTING PROMOTING SUBSTANCE EXTRACTED FROM *LENTINUS EDODES* CULTURE

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The crude extracts from *Lentinus edodes* mycelia culture (LEM; Noda Shokukin Kogyo Co. LTD.) enhanced the adventitious root formation in *Azukia* epicotyl cuttings. The rooting promoting activity was observed both in 80% ethyl alcohol soluble and insoluble fractions from the extracts (LEM). Partial purification of the insoluble fraction by column chromatographies proved the material to be composed of several amino acids. And the quantitative analyses were performed on the active compound, based on the studies with *Azuki* bean epicotyl cuttings. We observed extensive stimulation of the root formation of the cuttings upon exogenous application of the active compound.

MOLECULAR ANALYSIS OF EVENTS LEADING TO ADVENTITIOUS ROOT INITIATION IN SUNFLOWER

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In an attempt to isolate genes that are involved in the early stages of adventitious root formation we have taken a molecular approach to dissect the events that occur in hypocotyls after the excision of the entire root system from 6-day-old sunflower seedlings. Apart from a wound response, root excision in sunflower leads to the formation of primary phloem fibers and initiation of adventitious root primordia. Root primordia are clearly visible in the basal 2 cm of the cut hypocotyl at 24 hours after the removal of the primary root. Various hormone treatments affect the number and development of these primordia. Protein analysis reveals that 59 novel proteins are synthesized between 6 and 24 hours in the basal regions of the hypocotyl after derooting. Hypocotyl RNA isolated from 10 hours and 24 hours derooted seedlings was used to construct cDNA libraries. Analysis of *in vitro* protein synthesis patterns directed the use of subtractive probes for the isolation of cDNAs specific to the derooting initiated processes. Probes were constructed using either RNA from hypocotyl tissue at 0 hours, or cDNA derived from it, as the driver component of the subtraction. Several cDNA clones have been isolated from the screening of the libraries with these probes. Several represent mRNAs that appear to be associated mainly with the wounding response but others represent mRNAs that are not induced by wounding alone and thus are likely to be involved in root or phloem fiber formation. These cDNAs are currently being assessed for their responses to hormone treatment and to the tissue location of the corresponding messages.

CONFOCAL LASER SCANNING MICROSCOPICAL OBSERVATIONS OF ADVENTITIOUS ROOT FORMATION IN APPLE ROOTSTOCK M9

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The histological structure of living stem discs of apple rootstock Jork M9 was visualized using confocal laser scanning microscopy (CLSM). In epifluorescence mode the autofluorescence of unstained tissues could be utilized to image the highly fluorescent xylemic and phloemic areas while the nonfluorescent cambium appeared as a black ring. In time course experiments, discs were examined at several periods after a root induction treatment with IBA. Until up to 30 hours after IBA treatment no significant alteration in overall tissue morphology could be detected. Between 30 and 54 hours after IBA treatment a dramatic thickening of cambial tissue was observed. Twenty-four and 48 hours later the increase in cambial tissue had proceeded but now seemed to be restricted to certain regions which extended deeply into the cortical parenchym and represented the adventitious root meristems. However the woody parts of the discs and the pith were not affected by the meristematic outgrowth.

Digital image analysis of the time series lead to kinetic information about early phases of root outgrowth. Comparison of CLSM images with paraffin sections yields a possibility to rapidly and noninvasively analyse the kinetics of adventitious root initiation under different experimental conditions.

EFFECT OF DARK TREATMENT ON ENDOGENOUS IAA AND IBA LEVELS DURING ADVENTITIOUS ROOTING OF *MALUS* GROWN *IN VITRO*

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The effect of dark and light treatments on metabolism of IAA and IBA in IBA-treated M9 York apple shoots grown *in vitro* was studied. Both percentage rooting and number of roots per rooted shoot were greater in shoots placed in the dark for 4 days compared to those left in the light. Endogenous IAA and IBA concentrations were determined during the dark and light periods. Samples were purified using solid phase columns and HPLC. Quantitation was carried out by GC-MS. Concentrations of free and total IBA increased significantly during both light and dark treatments. Concentrations of free IBA increased from 40 ng/g FW to 300 ng/g FW in the light, but were greater than 400 ng/g FW in the dark. In addition, the concentrations of free IBA remained higher for a longer period of time in the dark. Increases in endogenous IAA in the dark were significantly less than those of IBA and were minimal compared to those in the light. Uptake of [^{3}H] IBA differed in the light and dark, with significantly greater uptake occurring in the dark. Conversion of IBA to IAA occurred in the dark, but the amount was relatively small compared to the total endogenous concentration of IAA. Results of these studies indicate that although the conversion of IBA to IAA does occur, and increased synthesis of IAA occurs as a result of treatment with IBA, the major effect on induction of rooting appears to be due to the IBA itself.

VARIATIONS IN POLYAMINE CONTENT DURING THE ROOTING PROCESS OF *VITIS* ROOTSTOCK 140 Ruggeri CUTTINGS

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To enhance the rooting process in woody cuttings some authors have treated the material with polyamines. These substances have been considered, by some authors, as markers of rooting in tissues cultured *in vitro*. Therefore, in *Vitis* rootstock 140 Ruggeri cuttings we wanted to study how the pattern of these substances changes during the rooting process.

For this we utilized experimental systems able to modify the rooting process timing, such as: time of collecting of the material during the year, dipping of the cuttings and cold (2°C) treatment. The results showed an increase in putrescine before the rooting process, presumably accompanying root primordia formation. The amount of putrescine decreases later with the growth of the roots. For spermidine and spermine it was not possible to substantiate variations linked to rooting. In this study we aimed to define the moment in which the rooting process requires an equilibrium Put/Spd + Spm > 1.

INDUCTION OF ADVENTITIOUS ROOTS *IN VITRO* BY TRIAZOLE GROWTH REGULATORS

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Triazole growth regulators (paclobutrazol, uniconazole) brought about an inhibition of shoot elongation, growth of lateral branches and formation of characteristic coral-like, fragile-looking lateral roots in intact seedlings of mothbean (*Vigna aconitifolia*) cultured on MS-media containing sucrose and gelrite. The roots proliferated and burst open to produce callus which developed green nodule-like structures. The treatment also induced profuse formation of adventitious roots on the hypocotyl as well as epicotyl. At a high concentration leaf abscision was also enhanced. In contrast, gibberellic acid promoted elongation of hypocotyl and epicotyl, but proved highly inhibitory for the growth of lateral roots. Simultaneous application of gibberellic acid with triazoles completely reversed the growth inhibition caused by paclobutrazol and uniconazole, but production of adventitious roots continued uninterrupted throughout the hypocotyl length. It appears that triazoles bring about the induction of adventitious roots in this system by influencing the levels of endogenous growth regulators, perhaps auxins.

ADVENTITIOUS ROOT FORMATION IN *RUMEX*: AN ADAPTIVE RESPONSE REGULATED BY AUXIN?

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Waterlogging of the root system of *Rumex* species (docks) causes the initiation of a very distinctive type of roots. The new roots originate from the base of the rosette-shaped shoot and the upper part of the tap root, and contain aerenchymatous tissue. Oxygen limitation in the soil is the key factor in this rooting process, and the effect of waterlogging can be mimicked by application of low oxygen pressures in the culture medium of plants on hydroculture. *R. palustris*, which occurs on wet hypoxic soils, develops high numbers of these adventitious roots, whereas *R. thyrsiflorus*, a species from dry and well-aerated substrates, shows only little adventitious root formation. In addition, the aerenchyma of the latter species is far less developed. The aim of our research is to determine what causes the differences in adventitious root formation of these two closely related species.

With both species, application of the auxin 1-NAA (naphthylacetic acid) to the leaves led to development of adventitious roots to a comparable extent as when root systems were treated with low oxygen pressures. Additionally, when roots were treated with low oxygen pressures in combination with application of NPA (naphthylphtalamic acid, a potent inhibitor of auxin transport) to the shoot, a strong inhibition of adventitious root formation was found. This suggests that auxin transport in the primary root system is reduced during hypoxic conditions, causing an accumulation of auxin at the base of the aerated shoot. The increase in auxin levels might well be the initiating factor in adventitious rooting.

Future research will be focused on possible differences in endogenous auxin concentrations or sensitivity towards auxin between the two species. In addition, preliminary results have shown that ethylene might play a substantial role in this rooting process as well.

ROOT REGENERATION IN *MALUS*: A MOLECULAR AND BIOCHEMICAL APPROACH

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A model system, based on stem discs from *in vitro* produced apple shoots allow us to study early events during root regeneration. The rooting process has been mapped anatomically. A protocol for *in situ* hybridization with probes derived from root specific cDNA clones is under development. The influence of root specific inhibitors on cell divisions, organisation of root primordia and outgrowth of the roots are also investigated.

INDUCTION OF ADVENTITIOUS ROOT FORMATION ON STEMS OF *PINUS SILVESTRIS* AND *PINUS MUGO* BY AIR LAYERING

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Experiments were performed on 8-year-old *Pinus silvestris* and 25-year-old *Pinus mugo* growing outdoor in the Experimental Station in Rogow, Central Poland. In spring (17th March) some of the 1-year-old stems from the middle part of the crown were girdled at a distance of about 10 cm from the apical buds. The injured (1-cm-long) and the adjacent portions of stem were enclosed with moisted peat and wrapped with black polyethylene film and aluminium foil. At the distal part of the girdle there appeared an abundance of callus tissue from which after ca. 3 months the adventitious roots emerged. Thirty percent of *Pinus silvestris* and 100% of *Pinus mugo* treated stems were rooted. The roots (4-5 per stem) appeared mostly at the lower parts of stems. However, the newly formed shoots, liberated from apical dominance, grew in the vertical direction. Rooted stems were cut off from the mother plants and planted in pots with mixture of peat and sand and placed in a greenhouse for further investigations.

ROOT SYSTEM DEVELOPMENT AND PLANT GROWTH

POSTER ABSTRACTS

AERIAL ROOT FORMATION IN *CISSUS MICROCARPA* VAHL. (VITACEAE)

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The process of aerial root formation was followed for a whole season in *Cissus microcarpa*, a woody vine growing in open sites in the tropical rain forest of S.E. Mexico. The effect of stem position (vertical *vs.* horizontal) and number of internodes on root formation was investigated. Cleared stem segments and anatomical sections were used to determine whether preformed adventitious root primordia existed in the stems, and the site of origin of these roots. It was found that neither stem position nor number of internodes were determinant in aerial root formation, since roots formed under all tested situations (from one to seven internodes long, lying either horizontally or vertically). Aerial root origin was traced to the wide medullary rays.

ROOT REGENERATION AND GROWTH RESPONSES OF BARE-ROOT SCARLET OAK SEEDLINGS TO TEMPERATURE, K-IBA, AND DCPTA ROOT PRIMING TREATMENTS

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Near the end of winter storage in a 4 ± 1°C cold room, root systems of bare-root *Quercus coccinea* seedlings were subjected to various combinations of 21 ± 3 and 4 ± 1°C root zone temperatures (0, 7, 14, and 21 days), K-IBA root dips (0, 1, 3, or 5 mg/liter, 15 sec.), and DCPTA [2-(3,4-dichlorophenoxy) triethylamine] root soaks (0, 1, 10, 100 mg/ liter, 30 min.) in an attempt to increase the number of adventitious roots regenerated from old roots present during storage. Most regenerated roots appeared to be adventitiously initiated from older suberized roots, primarily the taproots. The number of regenerated small roots (<1 mm diameter), large roots (≥1 mm diameter), and root weights increased for IBA-treated seedlings with increasing IBA concentrations and days in 21°C root zones. With DCPTA-treated seedlings the number of regenerated small roots, large roots and root weights increased slightly with increasing days in 21°C root zones, but not with increasing DCPTA concentrations. At budbreak, IBA treated seedlings held for 7 to 14 days in 21°C root zones had the greatest root regeneration, followed by 21 and 0 days in 21°C and root zones, respectively. Treatment effects on first year root and shoot growth following outplanting will also be discussed.

INVOLVEMENT OF CYTOKININS IN LATERAL ROOT DEVELOPMENT

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Application of zeatin to the tap root apex of *Pinus pinea* seedlings inhibited lateral root development while iso-pentenyladenine (2iP) increased it. The increment effect of 2iP application was caused by increasing the number and the average length of elongating lateral roots. Application of radiolabelled zeatin and 2iP to the tap root apex resulted in different distribution along the intact tap root. Zeatin was concentrated in the lower section (which does not contain lateral roots). On the other hand, 2iP was more mobile, and 3 hours after application, 30 percent of the radioactivity was found in the upper section of the root (which includes well-developed lateral roots). The results suggest that these two natural cytokinins might play a different role in the regulation of lateral root development.

MYCORRHIZA IN RELATION TO ROOT FORMATION

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The present paper focuses on influences of mycorrhizal association on formation of roots under stress conditions. Special emphasis is given to root formation at non-conventional sites.

Both ecto- and endomycorrhizal association at the seedling stage is reported to cause an early, better and faster development of primary laterals from the radicle. The linkage of mycorrhizal fungi with primary roots of germinating seedlings is an important feature in seedling establishment under stress conditions and at unconventional sites. Not only the growth of primary laterals, but the mycorrhizal fungi also influence the activity of root meristem, root hair formation and lateral root initiation, thus changing the potentials of root systems to exploit the whole soil profile to its maximum benefit.

The mechanism involved in the process of root initiation with mycorrhizal fungi is not yet clearly understood. Involvement of various growth hormones such as auxins and cytokinins is known in case of ectomycorrhizae. However such modifications with endomycorrhizae are attributed mainly to nutritional effects following greater phosphorus uptake. Still, direct detailed studies are required to unravel the basic mechanisms involved in mycorrhizae-induced changes in root formation.

ADVENTITIOUS ROOT FORMATION AS AN ADAPTIVE RESPONSE TO ENVIRONMENTAL STRESS

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In order to survive environmental stresses, plants have to develop morphological and physiological adaptations. In river areas throughout the world, stress may be caused by flooding ranging from waterlogging of the soil to submergence of whole plants. Long-term waterlogging negatively affects the amount of available oxygen. Short-term changing water levels may compact the soil and cause mechanical stress. Both hypoxia and mechanical resistance injure root systems. Our research line is to compare stress reactions in species of *Rumex* and *Plantago* occurring in flooded areas with those of related species from dryer environments. In contrast to high-elevation species, flood-resisant *Rumex* plants are able to develop new roots upon flooding. Most of these roots originate from the shoot/root junction and thus serve as an adventitious root system, that mainly occurs in the upper parts of the soil. Plants from flooded sites produce more new roots than plants from dry habitats. New laterals are characterized by aerenchyma, which has been proved to be an adaptive response to flooding. Flooding-tolerant plants have higher porosities than intolerant species. The functional meaning of aerenchyma is restoration of aerobic conditions, both within and around the root tissues. Upon submergence, tolerant plants respond with petiole and stem elongation aiming to restore plant-air contact. The hormone ethylene triggers this elongation. Leaf emergence induces the formation of the new roots. Soil compaction also determines root anatomy and patterns of root systems. Small soil pores may limit the formation of new aerenchymatous laterals. In *Rumex*, aerenchymatous roots collapse under increased pressure of the soil. In *Plantago*, some species are able to grow in wet and compacted soils. In these plants new porous roots are not affected by small confined soil-pore diameters.

The field distribution of plants in flooding gradients can, at least partially, be explained by their ability to form adventitious roots upon environmental stresses.

COMMERCIAL *IN VITRO* MICROPROPAGATION OF *MORUS ALBA* L. (MULBERRY)

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Sericulture development is affected by the cost of mulberry plantations. *In vitro* propagation of mulberry (*Morus alba* L.) may reduce these costs. Sterilization from winter buds was first carried out with 70% ethanol for 30', followed by 6% NaCl chlorine free and a few drops of Tween 20 for 20', buds were then rinsed three times in distilled sterile water. The shoots were maintained in Shenk and Hildebrand (1972) basal medium, Ba 0.5 mg/l, GA_3 0.5 mg/l, NAA 0.01 mg/l for the first 2 months. The buds were multiplied in DKW medium (1984), Ba 1 mg/l, IBA 0.01 mg/l. For rooting they were tested in MS and QL basal medium with 0.5-2 mg/l IBA and 50 mg/l activated charcoal added. Greenhouse acclimatization of the plants was tested in small tunnels covered with PVC plastic film for 40 days.

Rooting percentage of 100% was obtained for Kokuso 21 in a culture medium containing 1.5 mg/l MS, IBA and 50 mg/l activated charcoal. The best culture medium for Kayrio N. was found to be 3 mg/l QL, IBA provided a rooting percentage of 100%. It was noted that acclimatization of the plants was performed with a very high percentage (95%) of survival. The results confirm that propagation of certain mulberry cultivators, which are particularly adapted for silkworm rearing, may be possible.

ROOTING AND CONDITIONING OF COFFEE PLANTS REGENERATED *IN VITRO*

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Rooting is a crucial and important step in micropropagation. The success of any technique of *in vitro* plant propagation is closely linked to the percentage of survival of newly formed plantlets and on the ability of their root systems to acclimatize to the autotrophic conditions. The rooting in woody plants is very difficult and still one of the major constraints to their *in vitro* micropropagation. In commercial terms the process of *in vitro* rooting is very expensive and there are references in the literature to the weakness of the root systems produced *in vitro*. So, some authors search for practical and inexpensive techniques for *in vitro* rooting of the plants produced *in vitro*. In coffee, intensive work has been done to establish a practical method of *in vitro* and *in vivo* rooting of plantlets obtained using different techniques of coffee micropropagation. Different experiments were carried out to study the coffee rooting phenomenon *in vitro* and *in vivo*. The influence of factors like concentrations of mineral salts, levels of sucrose, effect of activated charcoal, growth regulators such as IAA, IBA, ZEA, BAP, and KIN were considered as well the influence of media consistency. Attempts were also made to induce rooting *in vivo* of newly formed plantlets. Histological sections and microscopical examinations were made to study the rooting and to establish differences in *in vitro* and *in vivo* rooting at the level of the vascular system. The influence of *in vitro* and *in vivo* rooting in plant acclimatization was also studied. Results concerning to these studies will be presented and discussed.

A METHOD FOR ANNUAL PLANT ROOT SYSTEM STUDIES IN THE FIELD BY ROOT MAPPING: ADVANTAGES AND PROBLEMS

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Root access and variability of spatial distribution are real problems for quantification of root systems in the field. Methods are still imperfect. Root length density (RLD) and distances (RD) are estimated by a root mapping method using the number of root intersections (N) per unit map area (S) in a soil profile. The relationship between N/S and RLD is not direct, but depends on theoretical and experimental calibrations, especially of root orientation in the soil. These calibrations have been studied with a corn root system in an Ivorian soil. Experimental results prove that the main advantage of root mapping, as compared to sampling methods, is to allow the study of spatial variability of root growth, related to soil constraints. This information is important to assess the soil volume open to root uptake by modelling. The main problem is the indirect estimation of the root system after calibrations, giving only semi-quantitative results. Choice between two methods depends on the target of best accuracy selected: mean root density or variability of this root density.

MICROPROPAGATION AND ROOTING OF FOREST BROADLEAVED SPECIES

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This consortium has identified elite trees of the following species and has established clone banks in nurseries as grafted plants and *in vitro*, *Castanea, Camellia, Betula, Acer, Alnus, Fagus, Fraxinus, Juglans, Prunus, Quercus, Ulmus, Tilia,* and *Syringa.*

Approaches to large scale propagation of elite material include:

Selection and Culture Initiation: Data bases are generated on elite trees. Shoot cultures have been initiated from elite germplasm of either mature or seed material.

Rejuvenation and Micropropagation: Methods of rejuvenation include epicormic shoots, etiolation, cascade grafting, root suckers, shoot forcing, controlled vitrification and explant orientation *in vitro*. These have facilitated culture initiation and micropropagation. Micropropagation procedures have been developed to the pilot scale for *Castanea, Quercus, Juglans, Camellia, Betula, Prunus,* and *Tilia* and are being advanced to the industrial scale. For the other species, micropropagation is at the laboratory scale and also somatic embryogenesis in *Camellia, Castanea, Prunus,* and *Quercus.*

Rooting, Weaning, Automation: Rooting is dependent on genotype and importantly on the physiological state of the shoots with regard to their degree of juvenility and the micropropagation stage. Several biochemical markers have been identified which can predict the rootability of shoots *in vitro*. Automated systems of plant handling and nutrient replenishment are studied.

Economics and Germplasm Conservation: The economic aspects of micropropagating hardwoods in combination with conventional cutting propagation using elite material is being studied. Several methods of conserving elite material *in vitro* either cryopreserved or with growth limiting conditions are being evaluated using elite stocks.

TRIAZOLES IN THE ROOTING MEDIUM AFFECT ROOTING AND EXTENT OF ROOT DEVELOPMENT IN *PRUNUS SEROTINA*

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Sometimes acclimatization is not successful in part due to poor root development. Since triazoles have been shown to increase root to shoot ratio and alter root development, they were evaluated as a supplement to the rooting medium to increase root growth and improve acclimatization. Root initiation and development of shoots with the triazoles, paclobutrazol (0.15 mg/L) or uniconazole (0.15 mg/L) in the presence of indolebutyric acid (0.5 mg/L), incorporated into the rooting medium were compared to those with indolebutyric acid (0.5 mg/L) alone, just prior to transfer to the greenhouse, to determine their effects on root development and subsequent survival. Rooting percentage, root fresh weight, and number were not affected significantly by either paclobutrazol or uniconazole treatments. Paclobutrazol and uniconazole decreased average root length and roots tended to be thicker. Four weeks after transfer out of the *in vitro* environment, there was a more advanced root system having numerous tertiary roots with triazole treatment and none with the IBA treatment. Plant quality was also improved by triazole treatment.

AUXIN EFFECTS ON ADVENTITIOUS ROOT FORMATION IN CONIFERS

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In our model system for studying root initiation in hypocotyl cuttings of *Pinus contorta*, up to 100% rooting can he achieved. The cuttings are cultured *in vitro* or *ex vitro* in liquid nutrient solution. The auxin treatment is usually a pulse treatment for 6 hours with 1.23 mM IBA. When cultured *in vitro*, without auxin treatment, the cuttings form roots originating from a wound tissue. The roots are usually visible after a month and several months are needed before maximal percentages of rooting are achieved. When the cuttings are cultured *ex vitro* usually no wound tissue is formed and the roots can be formed directly on the stem. After an auxin treatment maximal rooting is achieved within 3 weeks whether the cuttings are cultured *in vitro* or *ex vitro*. The auxin treatment also stimulates direct root formation, without previous formation of wound tissue. These different types of rooted cuttings were compared for growth capacity under optimal conditions. The relative growth rates (RGR) of wound tissue rooted cuttings were lower than for the other two types of cuttings. The difference in number of roots between auxin induced and spontaneous rooted cuttings (2 and 35) was of no importance for RGR under optimal growth conditions.

The pulse treatment with IBA was also tested in epicotyl cuttings of *Pinus sylvestris*. The epicotyl cuttings were cultured *ex vitro* in liquid nutrient solution. As in the hypocotyl cuttings, auxin stimulated fast root formation. The auxin stimulated early rooting (within 6 weeks) but did not stimulate late rooting (after 6 weeks). Roots originating from vascular tissues were found only in auxin treated, early rooted cuttings. The same rooting method was also used to induce rooting in adventitious shoots of *Pinus contorta*, formed on isolated zygotic embryos after BA treatment. Within 12 weeks 75% of the adventitious shoots had developed roots. In studies of growth capacity of the rooted adventitious shoots (plantlets) it was shown that the size of the plantlets was of importance for the RGR. The plantlets with the highest initial weight had the same RGR as seedlings.

In an ongoing project we are searching for "root inducing genes." We are comparing two cDNA libraries, one from root segments in which lateral root formation is occurring and another from segments in which the lateral root formation is completed (see Poster of Grönroos, Flygh, and Lindroth).

ADVENTITIOUS ROOTING AND GROWTH OF ASSOCIATED SHOOT BUDS ON STOLONS OF WHITE CLOVER

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In clonally growing white clover, adventitious roots may arise from the two primordia sited at each node. Each node also has an associated axillary shoot bud which may develop to form a new active meristem. In order to further understand the significance of root development for the growth of shoot buds we sampled field populations and described the state of shoot and root buds at each node using a combination of observation with an incubation technique to classify buds into a number of viability categories.

Viability of axillary buds was unaffected by root presence but as bud development progressed the presence of a root became increasingly important. Root presence was highly correlated with the establishment and survival of branches but was less closely related to branch initiation and was unrelated to the viability of axillary shoot buds. This association between nodal roots and branches was strongest when resources were limited. Although current opinion stresses the dependence of a branch on its nodal root, this study provides evidence that the probability of survival of a nodal root is dependent upon the success of development of the axillary shoot bud. During periods of intense root development and initiation in late winter-early spring, root presence and viability of axillary buds were inversely related. The physiology of this relationship is the subject of further study.

ROOT DEVELOPMENT INITIATED BY AUX GENES IN *BRASSICA* CROPS

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About 6 kb sequences of auxin genes in pRiA4, pRi1855, pTiAch5 and pTiTm4 were separately cloned in binary vectors and cointergrate vectors, and transformed into Chinese cabbage, cabbage, and cauliflower. All aux gene sets of different sources initiated roots on hypocotyl segments and cotyledon discs inoculated with the related agrobacteria. However, a slight or significant difference was observed in root induction frequency of the genes and root growth rate of the transformants. pRiA4 derived aux genes seemed to be mostly sensitive and induced more roots and transgenic plants, from which several trasgenic lines of Chinese cabbage and cabbage were screened with strong root systems and fastly grown leaf head and corm. Potential uses of *Agrobacterium* aux genes for crop improvement of important agromical traits is discussed.

EFFECTS OF AUXIN TRANSPORT INHIBITORS ON LATERAL ROOT DEVELOPMENT

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The number of emerged lateral roots and spacing between them can be modified by auxin treatments (Wightman *et al.* 1980, Physiol. Plant., 49, 304). With this intention, we have studied the effects of various auxin transport inhibitors, namely TIBA and some phytotropins (NPA, PCIB, DPX1840) on two different systems. At first, we have used adventitious roots obtained by *in vitro* culture of axillary buds of *Stachys silvatica* (6-week-old) and, furthermore, 6-day-old primary roots of *Pisum sativum.*

For *Stachys silvatica*, TIBA increases the number of adventitious roots' laterals and the one expressed on a length basis, with a bell shape correlation to concentration (optimum around 0.3-0.5 µM of TIBA added to the culture medium). This increase is particularly marked in the first apical mm of the adventitious roots and is related to a displacement of the root's ramification zone towards the root apex. The average spacing between two subsequent laterals, over the whole root, is also clearly reduced (from more than 60%, which is more than the adventitious root growth inhibition).

For *Pisum* ("Douce Provence"), there is rather little inhibition of root elongaion (except NPA, 30 µM and PCIB, 100 µM). The auxin-antagonists tested (0.1 to 100 µM) reduce the total number of laterals, also on a length basis (in agreement with Hinchee and Rost 1992, Bot. Acta, *105*, 121). The extent of the ramification zone is decreased up to half of the control and shows a basal displacement (*id est* away from the root apex, especially for the youngest laterals). So, at first glance, the average spacing is increased but is, in fact, unmodified versus control if yielded to the length of the ramification zone.

So, it seems obvious, even if the reactivity is different in the two systems, that auxin transport is involved in lateral root development, especially root emergence, and concerned in regulating the position and number of these laterals. These facts will be discussed in relation with the analysis

PRODUCTION OF ADVENTITIOUS ROOTS IN *PROSOPIS CINERARIA* AND *ALBIZIA LEBBECK* PLANTATIONS IN ARID INDIA

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Biomass of adventitious roots is important for cycling of nutrients and turnover of organic matter in plantations and agroforestry systems. The present paper quantifies the adventitious root biomass in 6-year-old plantations (5 x 5 m spacing) of *Prosopis cineraria* and *Albizia lebbeck*, two important multipurpose nitrogen fixing trees of arid India. In *Prosopis cineraria*, biomass was estimated to be 3.5 t/ha/y (21 replications of soil cores excavated during summer, winter and rainy seasons), and it was uniformly distributed throughout the soil column up to 1.5 m, while in *Albizia lebbeck* the biomass in adventitious root was almost double (6.4 t/ha/y), as compared to *Prosopis cineraria,* and 80% of it was estimated in top 30 cm soil. Lesser root biomass produced in *Prosopis cineraria*, and its uniform distribution in deeper layers could be one of the reasons that the yields of agricultural crops in general do not decline in association with this species, in contrast to *Albizia lebbeck* where maximum adventitious roots (80%) were confined to top 30 cm soil. The results are discussed from the point of view of combining agricultural crops with these two species in arid parts of India.

NUMBER OF ADVENTITIOUS ROOTS: INFLUENCE ON GROWTH FOR RUBBER MICROCUTTINGS

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Root expression on *Hevea brasiliensis* microcuttings produced through *in vitro* culture occurs within 3 to 5 weeks after root induction. One, 2, or 3 adventitious roots are emitted at the base of the stem, respectively in the proportion of 74, 24, and 2% of the rooted plantlets.

Growth kinetics of microcuttings with 1 or 2 adventitious roots ("pseudo taproots") have been compared during the acclimatization time (0-4 months after transfer out of tube) and after planting out in the field.

At the end of the acclimatization phase, aerial growth is significantly higher for plantlets with 2 pseudo taproots. One year after planting, the influence of the number of pseudo taproots on stem growth is inverted. This may be put in relation with the greater ability of unique taproots for geotropic growth, allowing a better exploration of the water rich deep soil layers during the dry season.

GROWTH KINETICS OF INDIVIDUAL ADVENTITIOUS ROOTS OF RUBBER TREE SEEDLINGS (*HEVEA BRASILIENSIS*) IN PLANTATION

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Observations on root systems of 0- to 18-month-old *Hevea* seedlings made in rubber tree plantations from the Ivory Coast reveal the existence of late initiated roots which are emitted at the base of the taproot, outside the acropetal branching sequence. These adventitious roots are emitted in successive waves and in a limited number are characterized by a specific morphology and a superficial plagiotropic growth.

Growth kinetics of adventitious roots from different emission waves have been observed on young *Hevea* seedlings in the field. Adventitious root length and shoot development of 4- to 11-month-old trees have been observed weekly during 13 weeks. Adventitious roots are emitted horizontally and deviate little from their emergence direction during the length of the experiment. Studying growth curves observed on these roots allows to distinguish between three main growth types conditioned by their initial growth rate: (i) indefinite periodical growth characterized by an important daily growth increment (8 mm/d), (ii) periodical growth with a slowing down occurring at variable times of elongation, (iii) definite and slow growth rate (1 to 2 mm/d). Moreover, the emission and the elongation periodicity of these adventitious roots seem to be closely related with the rhythmicity of shoot development.

Growth kinetics study of individual adventitious roots in plantation (dynamical approach) should help modelling superficial root system installation (see our other abstract in this volume) in close relation with shoot development.

POTASSIUM INDUCED ADVENTITIOUS ROOT FORMATION IN VIVIPAROUS HYPOCOTYLS OF *RHIZOPHORA APICULATA* BLUME

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The rooting potential in hypocotyls of *Rhizophora apiculata* Blume, as influenced by IBA and salts of potassium and chloride, was studied under laboratory conditions for 20 days. Potassium induced maximum rooting potential as well as in combination with IBA at lower concentrations. The different salts of potassium and chloride enhanced rooting in the order: - KCl > $MgCl_2$ > NaCl > $KN0_3$ > KH_2PO_4 > $CaCl_2$. Contents of potassium and chloride in leaves, root and growing medium were also analysed to elucidate the absorption and accumulation characters of the ions. The treated hypocotyls after analysis, were transferred to the field and were found well adapted to field conditions. Among the salts studied, KCl enhanced maximum length of roots, number of primary and secondary roots by 123 and 60% respectively. Such a maximum effect was not found with the salts of potassium and chloride when tested separately. The synergistic effect of potassium and chloride in viviparous seedlings of a tropical halophyte is discussed.

GROWTH OF THE ADVENTITIOUS ROOT SYSTEM OF THE RUBBER TREE (*HEVEA BRASILIENSIS*) IN PLANTATION

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Adventitious roots are an important component of the root system of the rubber tree (*Hevea brasiliensis*). They constitute, indeed, the scaffold of the superficial root system which extends in the first 30 cm layer of the soil. On seedlings, the first adventitious roots appear about 30 days after germination, near the base of the taproot (see our other abstract in this volume).

The growth of these adventitious roots has been studied in rubber plantations from Ivory Coast by the excavation of a great number of individual roots from the base (near the collar) to the apex, on trees of various ages. Our data showed the time-dependant variations of adventitious root length, and allowed us to model these variations by growth curves. From the model, it becomes possible to simulate and show graphically the extension rate and the spatial distribution of the root systems along time in plantation. These outputs are powerfull tools for plantation management.

COMPARISON OF ROOT SYSTEMS OF SEEDLINGS AND PLANTLETS IN *ACACIA SENEGAL* (L.) WILD.

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Comparative studies of radical systems from seedlings and plantlets of *Acacia senegal*, were made in *ex vitro* conditions using plastic minirhizotrons 95 + 30 + 4 cm, one side transparent (Riedacker 1974). The minirhizotrons were inclined 30-40% from vertical with the transparent side of the chamber out lower side. By this means the root systems could be observed as they adhered to the transparent side.

The regeneration and subsequent architecture of the root system was manipulated by pruning, which simulated damage done to typical root at the moment of transplantation. Roots were cut at one of two levels: (a) at 4 cm of base at neck level or (b) at 1.5 cm from the tip. The results show that for some treatments, once acclimation to transplanting is achieved, the rate of growth of the main root of the plantlets may exceed that of the tap root.

REGULATION OF *IN VITRO* ROOTING BY DIFFERENT CARBON SOURCES IN CORK OAK (*QUERCUS SUBER* L.)

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The influence of different carbon sources on *in vitro* rooting was studied with cork oak (*Quercus suber* L.) cultures. Sucrose, glucose, fructose, and the sugar alcohol sorbitol were investigated at a wide range of concentrations (10-60 g L^{-1}). The number and length of adventitious roots produced increased with increasing sugar concentrations. At the end of the rooting period (4 weeks), the percentage of rooting was 100% when glucose at 30, 40 or 60 g L^{-1} was used as the carbohydrate source. Rooting was completely inhibited by sorbitol. The shoot/root ratio of the plantlets produced decreased with the increasing concentration of sugar in the rooting medium. This study clearly shows that adventitious root formation is strongly dependent on carbohydrate supply and that there is an optimum level of carbohydrates for rooting. The mechanisms by which the carbohydrate source and concentration during rooting influence the process of plantlet acclimatization are unclear. The influence of *in vitro* preconditioning with carbohydrates (during the rooting stage on *in vivo* establishment of the plantlets produced will be discussed.

CARBOHYDRATE UPTAKE AND UTILIZATION DURING *IN VITRO* ROOTING OF CORK OAK (*QUERCUS SUBER* L.)

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In this study the influence of different carbon sources on sugar uptake and utilization was investigated in order to clarify the effect of increasing sugar concentration on rooting. The participation of sugars in the physiological/biochemical status of plantlets was determined on the basis of reducing sugar content and through the activity of acid invertase. Uptake of radioactive sugars from the medium and its distribution in the plantlets were also assessed. Reducing sugar content and acid invertase activity were measured in leaves and roots of plantlets rooted on medium containing sucrose, glucose, or fructose as carbon sources. Acid invertase was found to possess catalytic activity, whether or not the tissue was grown on sucrose-containing medium. The uptake and utilization of different carbon sources during *in vitro* rooting of cork oak and its contribution to plantlet development will be discussed.

ADVENTITIOUS ROOT FORMATION IN A DEVELOPMENTALLY DISABLED *PHASEOLUS* HYBRID

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Several investigators have reported that within the genus *Phaseolus*, adventitious roots occur spontaneously on hypocotyls of hybrid crosses between cultivars from different geographic origins (i.e., large-seeded cultivars of Andean origin and small-seeded cultivars of Mesoamerican origin). We are using this system to study the physiology associated with adventitious root formation. Measurements of shoot and root dry weight, total leaf area and number of primary root tips indicate that the hybrids begin to develop abnormally 2 weeks after germination. The primary root system begins to deteriorate coincident with the appearance of adventitious roots on the hypocotyls.

EFFECT OF AUXIN CONCENTRATION ON *EX VITRO* AND *IN VITRO* ROOTING OF *GMELINA ARBOREA* CUTTINGS

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Ex vitro and *in vitro* rooting was achieved for clonal propagation of *Gmelina arborea.* For *ex vitro* rooting, 10-cm-long stem cuttings from young shoots of 10-year-old trees were collected. The cuttings were treated with different concentrations (50-200 ppm) and combinations of auxins (IBA, IAA, and NAA). For each treatment 100 cuttings were immersed in aqueous solution of auxin for different periods (24, 48, 72, 69 hours). They were then taken out of the solution and implanted in a mixture of soil, sand, and sawdust (1:2:1) on a tray. Four to six strong roots were induced within 20 days in 80% cuttings when they were treated with 100 ppm each of IBA and NAA for 72 hours. It was followed by cuttings (75%) treated with 100 ppm each of IBA and IAA for 48 hours. For *in vitro* rooting, *in vitro* raised microshoots were excised from the culture flask and implanted individually on root induction medium. Within 3 weeks of implantation, 80% rooting was achieved on a root induction medium consisting of half strength MS salts supplemented with IBA and IAA at 1.0 mg/l each. In both the cases rooted plantlets were transplanted directly to clay pots containing sand, soil, and humas (1:2:1). The pots were bottom watered in the laboratory. Within 3-4 weeks the plants were large enough to transfer in the greenhouse. Sixty-five percent of the *ex vitro* rooted and 80% of the *in vitro* rooted plantlets survived.

TRANSFORMATION WITH *ROL* GENES IN KIWI AND CHERRY TO IMPROVE ROOTING, PLANT REGENERATION, AND CANOPY ARCHITECTURE

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Transgenic plants of two cultivars (Hayward and GTH) of Kiwi (*Actinidia deliciosa* A. Chev.) with *rol* A, B, C and *rol* B genes of *Agrobacterium rhizogenes* were obtained by a technique previously described (Rugini *et al.* 1991, Plant Cell Reports). The plants are in the field for agronomical tests. Transformation and plant regeneration of cherry (*Prunus avium*) rootstock F12/1, which shows difficulty in regenerating *in vitro*, were obtained by an original technique, that of taking advantage of the capacity of *rol* B to induce roots and the capacity of the species to regenerate *ex vitro* from root cutting or to produce root suckers. In this technique, transgenic roots were first induced by *Agrobacterium* carrying *rol* A, B, C genes, then were selected *in vitro* by kanamycin, and the plantlets were transferred to soil where, finally, the roots were stimulated to produce suckers.

Kiwi transgenic plants with *rol* A, B, and C showed a great root mass and an average number of internodes 78% superior to the control; an average area of each single leaf with shorter and larger section of petioles was 23% inferior. Rooting of leaf discs was almost 100% with a higher root number in a medium without auxin compared to 30-40% of the control. Also leafy semi-hard-wood cuttings of transgenic somaclones, placed into a heated bench, and basally treated with 2,000 ppm IBA dispersed in talc, rooted an average of 75% with 10 roots per cutting, versus 30% with 3 roots per cutting for the control. Transgenic *rol* B gene, leaf discs produced abundant roots in medium without auxin, and a low concentration of cytokinin (BAP) inhibited rooting in control leaf discs but not in transgenic ones.

The accumulation of polyamines (putrescine, spermidine, and spermine) in the leaves was equivalent to the control. The H^+-ATPase, observed by electron microscopy, showed an activity of 25% in *rol* A, B, C and 15% in *rol* B palisate and spongy cells, higher than control.

ROLE OF POLYAMINES AND THEIR INHIBITORS OF SYNTHESIS AND CATABOLISM ON ROOT INDUCTION IN FRUIT TREES

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Exogenous supply of polyamines (PAs), when combined with auxin, particularly putrescine and one product of their catabolism (H_2O_2), promoted earlier and higher rooting in some species, both *in vitro* and in cuttings, while in others it reduced it or had no effect. Olive, apple, and jojoba benefitted from putrescine treatments, while walnut, chestnut, pear, almond, and apricot showed no effect; furthermore, in pear they promoted a considerable leaf expansion. The positive effect on rooting was correlated to the endogenous synthesis capacity of the tissues, age of the explants, light intensity or dark conditions and period of treatment. In olive cuttings, putrescine increased rooting in all seasons, and in spring even increased it without auxin; the H_2O_2 had an effect when applied within 2 days from cutting preparation, while putrescine had an effect within only 1 day. Furthermore, putrescine also showed this effect *in vitro* when *Agrobacterium rhizogenes* was used in place of auxins in this species.

The PA synthesis inhibitors, DFMO applied singly at 1 mM, in olive, decreased rooting less than DFMA, but when they were applied together rooting was inhibited and Put or H_2O_2, singly or combined, partially reversed the inhibitory effect. Instead, the aminoguanidine increased rooting in olive, while the cycloheximide increased the root number in kiwi leaf callus.

Endogenous PA accumulation in olive explants, detected during the root induction phase, peaked at day 2 and the amount, form, and quality of PAs varied according to the capacity of the explants to root. It seems that in conditions of low or nil rooting all three PAs (putrescine, spermidine, and spermine) accumulated in bound form. At day 5, all PAs accumulated only in low concentrations in both kinds of explants (capable and incapable of rooting).

EFFECT OF SOURCES OF NITROGEN AND CORRELATION WITH CHANGES IN pH OF NUTRIENT MEDIA ON *IN VITRO* ROOT INITIATION AND DEVELOPMENT IN MICROCUTTINGS OF JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS* LAM.)

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Microcuttings of jackfruit were placed for rooting in medium containing half-strength MS with 10^{-6} M IBA. Variations were made in the supply of nitrogen by omitting NH_4^+ and/or NO_3^- in this medium. When nitrogen was supplied as both NH_4^+ and NO_3^- (standard half-strength MS), good initiation and macroscopic development of roots were obtained. When the sole source of nitrogen was NO_3^-, root primordia were initiated, as shown anatomically by fluorescence microscopy, but rarely developed. When the sole nitrogen source was NH_4^+, rooting depended on the form in which it was supplied: $(NH_4)_3\ C_6H_5O_7$ (ammonium citrate) promoted root development but NH_4Cl rarely gave macroscopically visible roots. This effect of NH_4Cl was pH-related: in unbuffered media containing NH_4Cl as the sole source of nitrogen, the pH dropped quickly to ca. 4.4 irrespective of the starting pH (4.5, 5.7, or 6.5) and, although some cell division could be observed by fluorescence microscopy, initiation of root primordia was significantly inhibited. When media with NH_4Cl were buffered, good rooting was obtained at pH 6.5. At pH 4.5, no microcuttings rooted and most showed only slight signs of cell division when examined anatomically.

INHIBITION OF ROOT ELONGATION AND LATERAL ROOT FORMATION IN CORN BY ANALOGUES AND INHIBITORS OF POLYAMINE BIOSYNTHESIS

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We have previously studied polyamine metabolism with respect to development of two root types: adventitious roots (Friedman *et al.* 1982, 1985), and the main embryonic root with its lateral roots (Schwartz *et al.* 1986). The corn root model permits the study of two facets of root morphogenesis: meristematic activity and cellular differentiation leading to root elongation on one side, and formation of lateral roots on the other.

L-canavanine, an arginine analog and a competitive inhibitor of arginine decarboxylase (ADC), inhibited considerably both primary root elongation and lateral root primordia of corn. This was accompanied by inhibition of mitosis, DNA synthesis, ADC and ODC activity, and spermidine accumulation. Addition of the polyamine precursors arginine and ornithine, but not of polyamines, abolished the aforementioned inhibition. Similar effects were found with MGBG, the SAM-decarboxylase inhibitor, and with cyclohexylamine, a spermidine synthase inhibitor.

EXPRESSION OF THE ROOT-SPECIFIC *parA* PROMOTER FROM *NICOTIANA TABACUM* IN TRANSGENIC *ZEA MAYS*

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We have developed a transient gene expression assay system in maize roots to identify promoters suitable for establishing high levels of root-specific expression of agronomically beneficial genes in transgenic maize. We have examined several available promoters and focused on one in particular, namely, the parA promoter from *N. tabacum* described by Takahashi *et al.* (PNAS *86*: 9279-9283). Our tobacco-derived parA promoter is 98.7% identical by sequence to that reported by Takahashi *et al.* We verified that the parA promoter fused to the GUS coding sequence controls root-preferred expression in stably transformed tobacco. In the maize transient assay, constructs with the parA promoter fused to either GUS or luciferase reporter genes were functional in 3-day-old to 2-month-old maize primary and adventitious root tissues with little or no activity in scutellum, coleoptile, or leaf tissues. We have also fused this promoter to the coding sequence of three *trans*-acting factors and enhanced activity of a luciferase reporter gene nearly 200-fold while maintaining root-preferred expression. We are currently assessing the reported role that auxin may play on the parA promoter in maize cells and examining the expression patterns of this promoter in stably transformed maize plants.

INTRODUCTION OF *ROL* GENES IN ROOTSTOCKS OF ROSE

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This research aims at the production of genetically modified rose rootstocks with improved growth performance under low energy conditions. Root growth and branching incidence are rootstock characteristics which are both under hormonal control and responsible for water and nutrient uptake. Cytokinins, produced in the root tips, affect the outgrowth of lateral buds of the scion. By the introduction of the *rol B* and/or *rol C* gene from *Agrobacterium rhizogenes* an altered hormone production of the roots is pursued. Since this genetic modification is only required for the roots, a method for transformation/adventitious root formation was developed. Adventitious roots were induced on stem slices of micropropagated plants incubated on IBA supplemented medium. Upon *Agrobacterium* infection, selective outgrowth of transgenic roots was obtained on kanamycin containing medium.

Physiological studies on the altered hormone metabolism of the transgenic rootstocks are underway.

PHOTOSYNTHESIS, WATER RELATIONS, AND DRY MASS AND NUTRIENT PARTITIONING DURING ROOT INITIATION AND DEVELOPMENT OF POINSETTIA CUTTINGS

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Gas exchange, water relations, dry mass and nutrient partitioning of apical stem cuttings of two poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) cultivars were studied during the initiation and development of adventitious roots. Net photosynthesis (Pn), and stomatal conductance (g) in cuttings were initially low. Upon formation of root primordia (2 days before visible root emergence), g increased and preceded significant increases in Pn. On the first day of root emergence, both Pn and g increased dramatically. Root initial formation and primordia development were independent of Pn, while Pn and g increased with root primordia development and emergence of adventitious roots. Foliar osmotic potential was highest upon formation of root primordia. Foliar relative water content (RWC) steadily declined, but increased rapidly upon formation of root primordia. Increases in dry mass and leaf area occurred immediately after sticking cuttings; greatest growth enhancement occurred at 100% rooting. Physiological and growth responses were similar for both cultivars. Fe, B, Zn, and Mo accumulated at the base of cuttings prior to root initiation. After root primordia development and root emergence, tissue elemental concentrations of the four micronutrients and Mg increased in the cutting base of both cultivars as did N, P, and K in the better rooting cv. Lilo. This research supports the hypothesis that the organization and emergence of adventitious roots stimulates photosynthesis. There appears to be no benefit from using higher light intensities during commercial poinsettia propagation until after poinsettia cuttings have initiated roots.

PACLOBUTRAZOL AND GIBBERELLIN DIFFERENTIALLY AFFECT ADVENTITIOUS ROOTS OF *FICUS BENJAMINA* AND LATERAL ROOTS OF CORN (*ZEA MAYS*)

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Practical aspects of commercial production of bonsai-like *Ficus benjamina* pot plants, using paclobutrazol treatment of rooted cuttings, have been discussed elsewhere. In the following, we present comparative data on the effect of paclobutrazol and gibberellins on adventitious and lateral roots of *Ficus benjamina* cuttings and corn seedlings, respectively, grown in nutrient solutions.

Paclobutrazol inhibited, and GA_3 promoted, elongation of adventitious and lateral roots of *Ficus benjamina* plants, but both growth regulators did not affect initiation of lateral roots on adventitious roots. Scanning electron microscopy revealed a marked reduction in cell length and width, and in cell wall thickness, of paclobutrazol-treated roots. Only cell length was promoted by GA_3 treatments. In corn, paclobutrazol inhibited both elongation of the main root, and lateral root formation on the main root. The inhibition was dependent on the time of application (0 to 140 hours) and could be reversed, partially, by GA_3. Inhibition of lateral roots probably resulted from the effect of paclobutrazol on the main root, and not on initiation of lateral root primordia.

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Our job at the North Central Forest Experiment Station is discovering and creating new knowledge and technology in the field of natural resources and conveying this information to the people who can use it. As a new generation of forests emerges in our region, managers are confronted with two unique challenges: (1) Dealing with the great diversity in composition, quality, and ownership of the forests, and (2) Reconciling the conflicting demands of the people who use them. Helping the forest manager meet these challenges while protecting the environment is what research at North Central is all about.

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